SINS

XVI Meeting
Italian Society for Neuroscience
Cagliari - October 8-11, 2015

Lecture 1

HUNTINGTON, THE STORY OF AN ANCIENT GENE IN SEARCH OF A BETTER FUTURE

Cattaneo Elena

Department of Biosciences - University of Milan, Italy

Huntingtin (htt) is the ~800 million-year old protein product of the Huntington's disease (HD) gene. The gene contains a polymorphic tri-nucleotide CAG repeat that is translated into polyglutamine amino acid (polyQ) residues in the protein. When this polyQ stretch at the 18 aminoacid (aa) position of the protein expands to over 36 residues, HD occurs, a fatal, genetically dominant, neurodegenerative disease. The CAG repeats are well conserved in deuterostomes, which suggests that they are an ancestral feature retained during the evolution of the protein. Htt carries a number of specific activities in the adult brain; for instance, it promotes transcription of neuronal genes among which is the BDNF, a neurotrophin critical for the survival and activity of cortical and striatal neurons that degenerate in HD. This presentation will highlight the power of combining evolutionary and developmental approaches to the study of the biology of disease genes and will review the more recent discoveries of the function of htt in the developing and mature brain.

Lecture 2

HOW WE SEE AND HEAR STUFF: VISUAL AND AUDITORY ROUTES TO UNDERSTANDING THE MATERIAL PROPERTIES OF OBJECTS

Goodale Melvyn A.

The Brain and Mind Institute - The University of Western Ontario - London ON N6A 5B7 Canada

Almost all studies of object recognition, including those using brain imaging, have focused on the geometric structure of objects (i.e. 'things'). Until very recently, little attention has been paid to the recognition of the materials from which objects are made (i.e. 'stuff'), information that is often signalled by surface-based visual cues (the sheen of polished metal) as well as auditory cues (the sound of water being poured into a glass). But knowledge about stuff (the material properties of objects) has profound implications, not only for understanding what an object is, but also for the planning of actions, such as the setting of initial grip and load forces during grasping.

In recent years, our lab has made some headway in delineating the neural systems that mediate the recognition of stuff (as opposed to things), not only in sighted people but also in blind individuals who use echoes from tongue clicks to recognize the material properties of objects they encounter.

I will discuss evidence from both behavioural studies and fMRI demonstrating that lateral occipital regions in the ventral stream play a critical role in processing the 3-D structure and geometry of objects, whereas more anteromedial regions (particularly areas in the parahippocampal gyrus and collateral sulcus) are engaged in processing visual and auditory cues that signal the material properties of objects.

Lecture 3

DOPAMINE AND SYNAPTIC PLASTICITY: FROM MOTOR TO COGNITIVE DYSFUNCTIONS

Calabresi Paolo

Clinica Neurologica, Università degli Studi di Perugia, Perugia, Italy

The striatum and the hippocampus play distinctive roles in memory processes via a critical synaptic integration between dopamine (DA) and glutamate inputs. Operative (striatal-dependent) and declarative (hippocampal-dependent) memory systems may act independently. These systems, however, can also be engaged to function in parallel as part of a dynamic system to integrate previous experience and adjust behavioral responses. In these structures the formation, storage, and retrieval of memory require a synaptic mechanism that is able to integrate multiple signals and to translate them into persistent molecular traces at both corticostriatal and hippocampal synapses. The best cellular candidate for this complex synthesis is represented by long-term potentiation (LTP). A common feature of LTP expressed in these two memory systems is the critical requirement of convergence and coincidence of dopaminergic and glutamatergic inputs to the dendritic spines of the neurons expressing this form of synaptic plasticity

Corticostriatal transmission is essential in the regulation of voluntary movement, in addition to behavioral control, cognitive function and reward mechanisms. LTP and long-term depression (LTD), the two main forms of synaptic plasticity, are both represented at corticostriatal synapses and strongly depend on the activation of DA receptors. Feed-forward and feed-back mechanisms involving striatal interneurons operate in association with striatal spiny neurons and endogenous DA to influence the formation and maintenance of both LTP and LTD.

The impairment of these two forms of synaptic plasticity in the striatum could account for the onset and the progression of motor and cognitive symptoms of Parkinson's disease (PD), characterized by the massive degeneration of DA neurons. In fact, both LTD and LTP are peculiarly controlled and modulated by dopaminergic transmission coming from nigrostriatal terminals. Levels of endogenous DA influence the threshold for the induction of synaptic plasticity.

PD patients show impairments in multiple cognitive performances even at the early stage of the disease. An alteration of hippocampal LTP represents the major candidate for explaining learning and memory deficits in PD. Accordingly, CA1 hippocampal LTP is altered in both neurotoxic and transgenic models of PD and this plastic alteration is associated with an impaired dopaminergic transmission and an altered subunit composition of synaptic NMDA receptors. Interestingly, the DA precursor L-DOPA was able to restore hippocampal synaptic potentiation via D1/D5 receptors and to ameliorate the cognitive deficit in parkinsonian animals suggesting that DA-dependent impairment of hippocampal LTP may contribute to cognitive deficits in PD patients.

Lecture 4

BRAIN CONNECTOMICS: EXPLORING THE CONNECTOME AND SYNAPTOME

DeFelipe Javier

Laboratorio Cajal de Circuitos Corticales (CTB), Universidad Politécnica de Madrid and Instituto Cajal (CSIC), Madrid, Spain

The principal goal in neuroanatomy is to define the detailed structural design of the nervous system. This challenge is one of the first steps towards understanding how neural circuits contribute to the functional organization of the nervous system, both in health and disease. The main difficulties involve unraveling the extraordinary complexity of the nervous system and to define how information flows through this finely organized synaptic network.

Over the years, neuroanatomy has evolved considerably thanks to the use of classical techniques and the introduction of new procedures. The term “connectome” has recently been proposed to refer to the highly organized connection matrix of the human brain, in analogy to the human genome. However, defining how information flows through such a complex system represents so difficult a task that it would seem unlikely it could be achieved in the near future, or, for the most pessimistic, perhaps never.

Circuit diagrams of the nervous system can be considered at different levels, although they are surely impossible to complete at the synaptic level. Even for a small mammal like the mouse it is impossible to fully reconstruct the brain at this level (we would need over 1.4 x 109 sections to fully reconstruct just one mm3 of tissue). Therefore, complete reconstructions of a small region of the mammalian brain are feasible, while structures like the cerebral cortex cannot be fully reconstructed.

Despite the technical difficulties, by adopting appropriate strategies with the tools now available coupled with the development of huge international projects, it should be possible to make spectacular advances in unraveling brain organization, even in humans. Indeed, advances in our capacity to marry macro- and microscopic data may help establish a realistic statistical model that could describe connectivity at the ultrastructural level, the “synaptome”, giving us cause for optimism.

Lecture 5

IMMUNE PROTEINS IN BRAIN DEVELOPMENT AND SYNAPTIC PLASTICITY

Matteoli Michela

CNR Institute of Neuroscience and Humanitas Research Hospital Rozzano, Milano, Italy

Since the neurodevelopmental hypothesis of schizophrenia, the pathophysiological origins of many neuropsychiatric diseases are increasingly recognized to associate with environmental influences. In the last years evidence accumulated indicating that inflammation, influencing the physiology and pathology in the immature and mature brain, can modify the risk and/or severity of a variety of brain diseases. Inflammation may resolve without any harmful effects on the brain, even contributing to reparative processes, or can be shifted to a chronic state, thus contributing to injury, enhancing CNS vulnerability and/or adversely affecting brain development. Although a number of molecules involved in inflammation have been found to regulate specific neuronal processes, the possibility that inflammatory cascades, either alone or in combination with a susceptible genetic background, may impact synapse formation and plasticity, thus leading to a disease condition, has not been addressed in a systematic way.

The talk will report a series of evidence indicating that immune challenges, prenatally or postnatally delivered, impact synaptic protein networks, thus resulting in neuronal modifications typical of psychiatric diseases. Results from these studies will allow the identification of new targets suitable for innovative therapeutic intervention.

Lecture 6

DOPAMINE AND REWARD: THE NEVER ENDING STORY

Di Chiara Gaetano

Department of Biomedical Sciences, University of Cagliari, Italy

The notion that links dopamine (DA) to reward stands as one of the most popular, yet most debated issues in neuroscience. Evidence for such a role was initially provided, almost 40 years ago, by experimental studies on intracranial self-stimulation (ICSS). The role of DA in ICSS reward was later extended to all rewards, conventional and pharmacological. Correlative evidence by a variety of techniques (extracellular recording, microdialysis, voltammetry and PET) also showed that activation of mesolimbic DA transmission is associated with reward and with stimuli conditioned to it.

Recently, optogenetic studies have shown that selective light driven stimulation of mesolimbic DA neurons projecting to the nucleus accumbens (NAc) is able of inducing place preference and of maintaining instrumental responding with characteristics superimposable to those of classical ICSS in a DA D1 and D2 receptor dependent manner.

These observations indicate that DA is linked to reward, although of a kind different from conventional consummatory reward (e.g. taste reward), not only post hoc, as a consequence, but also propter hoc, as a cause. The NAc, however, is an heterogeneous structure, being made of two subdivisions, the shell and the core, with different and eventually opposite functions. Microdialysis studies show that all drugs of abuse increase extracellular DA preferentially in the NAc shell. Lentiviral-siRNA-induced silencing of D1 receptor expression in the NAc shell but not core prevents acquisition of cocaine but not heroin i.v. self-administration. However, blockade on NAc shel DA D1 receptors impairs acquisition of morphine-conditioned place preference. Thus, NAc shell DA plays a role in drug reward and in acquisition of pavlovian incentive stimuli conditioned to the drug (pavlovian incentive CSs) and depending on the drug and the behavioral paradigm, is critical for each of these aspects. In rats responding for sucrose pellets by nose poking, extracellular DA increases selectively in the NAc shell without habituation as a result of the action of sucrose-conditioned incentive stimuli (sucrose CSs).

Drugs of abuse resemble those CSs in their ability to preferentially stimulate NAc shell DA. However, while drugs of abuse stimulate NAc shell DA unconditionally, incentive sucrose-CSs have to be reinforced to maintain their shell DA stimulant properties. Thus, drugs of abuse are unconditional surrogates of conventional incentive CSs. As such, their rewarding and incentive learning effects are not subjected to extinction nor to blockade by associated CSs. These differences are hypothesized to be critical for the ability of drugs of abuse to induce addiction.

SYMPOSIA

Symposium 1

NEUROADAPTIVE MECHANISMS IN ETHANOL WITHDRAWAL: FROM SPINES TO SPIKES.

Diana Marco

'G.Minardi' Laboratory of Cognitive Neuroscience - Sassari - Italy

Neuronal remodeling and culling are hypothesized to confer resilience to addictive-like behaviors, such as excessive ethanol drinking and dependence. Indeed, structural abnormalities are likely to contribute to synaptic dysfunctions which underlie the appearance of alcohol withdrawal signs and symptoms, that occur when suddenly ceasing the use of alcohol, thus perpetuating the addictive cycle.

We sought to investigate possible alterations produced by ethanol withdrawal on mesocorticolimbic transmission by exploring critical elements whose presence is strictly correlated with DAergic and GLUergic function, respectively: TH- and dopamine transporter (DAT)-positive fibers and postsynaptic density 95 (PSD-95). Spine density, morphology, and morphometry of MSNs in the Nacc shell were also investigated to obtain structural insights into pre- and postsynaptic elements of the triad simultaneously. We also performed patch-clamp experiments in Nacc shell slices obtained from ethanol-withdrawn rats to evaluate whether long-term depression (LTD) formation and its underlying synaptic currents are modified by experimental conditions.

Morphological, electrophysiological and behavioral methods.

Ethanol-dependent rats display a loss of dendritic spines in medium spiny neurons of the Nacc, accompanied by a reduction of TH-positive terminals and PSD-95 positive elements. Further analysis indicates that ‘long thin’, but not ‘mushroom’, spines are selectively affected. In addition, patch-clamp experiments from Nacc slices reveal a hampered LTD formation accompanied by parallel changes in field potential recordings and a reduction in AMPA-mediated synaptic currents. These changes are restricted to the withdrawal phase of ethanol dependence suggesting their relevance in the genesis of signs and/or symptoms affecting ethanol withdrawal, and thus the whole addicting cycle.

Overall these results highlight the key role of spine function on the evolution of alcohol dependence and suggest that the selective loss of ‘long thin’ spines may affect learning dysfunctions and significantly contribute to further ‘impoverish’ the already deficient dopaminergic transmission whose hypofunctionality is a major factor for the emergence of the harmful consequences of alcohol abuse/dependence.

GABA MECHANISMS IN ALCOHOL-INDUCED EFFECTS AND NEUROPLASTICITY

Korpi Esa, Vashchinkina Elena, Linden Anni--Maija, Vekovischeva Olga, Hyytiä Petri

Department of Pharmacology, Faculty of Medicine, FI-00014 University of Helsinki, Helsinki, Finland

Ethanol has many targets in the central nervous system, one of them being the γ-aminobutyric acid (GABA) neurotransmission system that mediates synaptic phasic and extrasynaptic tonic inhibition. Putative alcohol antagonist Ro 15-4513 is known to act on synaptic GABA-A receptors at γ2 subunit-dependent benzodiazepine sites (inactivated in γ2F77I transgenic mice) and has been suggested to act also at a putative δ subunit-dependent Ro 15-4513/ethanol binding site (inactivated in GABAA receptor δ subunit knockout mice), apparently at extrasynaptic sites.

However, antagonism of ethanol-induced sedation by Ro 15-4513 was unaffected in δ-KO mice, but blocked in the γ2F77I mice, suggesting that Ro 15-4513 action on this ethanol-affected behavior is mediated synaptic benzodiazepine sites (Linden et al., Front Neurosci 2011).

A strong injection stress-related reduction in 2-h limited access drinking of 10% ethanol was observed in γ2F77I and control mice, with a slight further reduction by Ro 15-4513.

No significant effects were observed by saline or Ro 15-4513 in δ-KO and control mice, which mouse lines had similar baseline alcohol drinking levels. In the VTA, a single dose of ethanol induced glutamate neuroplasticity in dopamine neurons of midbrain slices at 24 h after administration, as do many other drugs of abuse including the benzodiazepines diazepam, midazolam and zolpidem. Interestingly, behavioral effects of ethanol resemble those of the neurosteroid agonist ganaxolone and GABA-A agonist THIP, which target especially the extrasynaptic δ subunit-containing GABA-A receptors. Importantly, these compounds also induced a similar glutamate neuroplasticity in VTA dopamine neurons 1-6 days after a single dose. However, these compounds were poorly rewarding in mice, while alcohol induces both self-administration and place preference.

Our results indicate that ethanol has different effects on neuroplasticity and conditioned behavior than extrasynaptic GABA-A agonists, and that some effects of ethanol can be actually reduced by inhibition of synaptic GABA-A receptors. These results may be more in line with ethanol acting on synaptic GABA release than directly facilitating GABA-A receptor functions. Therefore, multiple presynaptic receptor mechanisms, such as opioid receptors and GABA-B receptors, might be more important as targets for treating alcohol-related behaviors than the widespread GABA-A receptor system.

INCREASED ALCOHOL CONSUMPTION AND SYNAPTIC PLASTICITY IN MOUSE MODELS OF EARLY LIFE STRESS

Sanna Enrico[1], Talani Giuseppe[2], Licheri Valentina[1], Gorule Ashish[1], Biggio Giovanni[1]

[1]Dept Life and Environmental Sciences, Sect Neuroscience and Anthropology - University of Cagliari - Cagliari - Italy, [2]Institute of Neuroscience, C.N.R. - Cagliari - Italy

A great body of experimental evidence suggests that perinatal brain plasticity increases the vulnerability to early life adverse experiences, which may lead to long-lasting abnormal development and behavior, including a higher risk for mood and anxiety disorders and abuse of psychoactive drugs including ethanol (EtOH). Despite many data are available that link early life stress and alcohol abuse vulnerability, the neurobiological mechanisms that result in an increased risk of addiction remain poorly understood. Different rodent models of early life stress have been established, including the post-weaning social isolation (SI), that are useful to further examine the relationship between prolonged postnatal stress and addiction vulnerability.

Our previous work showed that post-weaning SI in mice is associated with an enhanced voluntary EtOH intake, decreased plasma and brain levels of neuroactive steroids, and an up-regulation of extrasynaptic GABAA receptors in dentate gyrus (DG) granule cells compared to group-housed (GH) mice. In the present study, we examined the effects of SI on neuronal excitability and long-term plasticity of GLUergic synapses in different sub-regions of the hippocampal formation.

Socially isolated (SI) male C57BL/6J mice were raised individually for 6 weeks starting from weaning (PND21), whereas group-housed (GH) control mice were kept in cohorts of 4-6 per cage. Patch-clamp and extracellular recordings were performed in hippocampal slices in order to measure GABAergic and GLUergic currents as well as NMDA receptor-dependent LTP in response to HFS.

SI reduced LTP in CA1 pyramidal cells and EtOH was more effective in inhibiting LTP in SI than in GH mice. Current-clamp analysis of DG granule cells revealed a decreased in membrane input resistance and firing of action potentials in SI vs. GH mice, suggesting an inhibitory action on neuronal excitability. Voltage-clamp recording of GLUergic sEPSCs in DG granule cells revealed a decreased in frequency, as well as a shift from PPD to PPF, indicative of a reduced probability of GLU release. Since daily administration of progesterone during the 6-weeks of SI period reverts the changes in GABAergic tonic currents, LTP, and neuronal excitability caused by SI, it is likely that such effects associated with SI my be triggered, at least in part, by the decrease of plasma and brain levels of neuroactive steroids.

The latter effect, in turn, may drive the up-regulation of extrasynaptic GABA-A receptors in DG granule cells, and consequently the reduced excitability of the whole hippocampal excitatory circuitry.

Funded by RAS LR 7/2007, 2010.

ROLE OF THE OPIOID SYSTEMS IN ALCOHOL ADDICTION: RECENT EVIDENCES

Romualdi Patrizia, Caputi Francesca, Palmisano Martina, Candeletti Sanzio

Dept Pharmacy and Biotechnology University of Bologna - Bologna - Italy

Alcohol abuse is a chronic disorder representing a worldwide health, social and economic problem in modern society. Several studies focused on the understanding of how alcohol modulates different systems that are crucial for neuroplasticity. Clinical and preclinical studies showed that chronic alcohol exposure causes widespread changes in gene expression in human and animal brain and many of these changes seems to mediate the cellular adaptation leading to addiction.
In recent years, a great interest has been focused on the role of epigenetic processes in alcohol-induced effects on the central nervous system. In particular, it is known that alcohol influences DNA methylation, promotes the histone acetylation and modulates the activities of the enzymes that add acetyl groups (histone acetyl transferases HATs) or remove acetyl groups (histone deacetylases HDACs). Hence, alcohol affects the regulation of the gene expression of several systems, including the endogenous opioid system and the brain-derived neurotrophic factor (BDNF).
The aim of our studies is to gain more knowledge on alcohol-induced neuroplasticity by means of both in vitro and in vivo approaches investigating opioid gene expression and their epigenetic regulation in different models of alcohol addiction.

For the gene expression studies, treatments were conducted both on cell cultures and rats. RNA was extracted from cells or brain areas and then subjected to real time qPCR analysis.
For epigenetic studies, histone modifications, HDAC levels, as well as proteasome activity and its specific subunits gene regulation were analyzed in the different experimental conditions.

Results showed alterations of prodynorphin and nociceptin systems in the different phases of ethanol exposure, with a relevant role likely played in the amygdala complex and in the prefrontal cortex. In these CNS areas we also found a linkage between gene expression alterations and epigenetic modulation at pronociceptin and prodynorphin promoters following alcohol treatment.

Our data showed that ethanol exposure affects proteasome activity and its subunit gene expression thus strengthening the hypothesis that 26S-proteasome machinery could represent a target of alcohol effects.
In addition, our results demonstrate that ethanol induces selective epigenetic changes, thus better defining the role of opioid peptides in the ethanol-induced effects in the amygdala complex.
In conclusion, our findings could help to the understanding of how alcohol differentially affects the opioid systems in the brain and also suggest the dynorphin and nociceptin systems as possible targets for the treatment and/or prevention of alcohol dependence.

MEDICATION IN THE TREATMENT OF ALCOHOL USE DISORDERS: ROLE OF SODIUM OXYBATE AND BACLOFEN

Addolorato Giovanni

CATHOLIC UNIVERSITY OF ROME - Alcohol Addiction Unit, Department of Internal Medicine, Rome - Italy

Pharmacological approaches represent an effective strategy in the treatment of alcohol abuse and alcoholism. The efficacy of the drug in the management of both alcohol withdrawal syndrome and relapse prevention should entail a vastly simplified pharmacotherapy of alcohol dependence.

The present lecture will be focused on the main GABABergic drugs, gamma-hydroxybutyric acid (GHB) and baclofen.

GHB suppresses alcohol withdrawal syndrome (AWS) in alcoholics, with an efficacy similar to that of diazepam and chlormethiazole. Several studies have shown that 50-100 mg/kg of GHB, in 3 daily administrations, reduces the number of daily drinks and alcohol craving, and improves the abstinence rate. Non-responders benefited from a divided dosing schedule. Craving for GHB with consequent abuse during treatment ranged from 2.6% to 10.1%. Pharmaco-surveillance indicates that GHB has limited abuse potential in a clinical setting under strict medical surveillanceRecent studies have suggested that baclofen, the prototypic GABAB receptor agonist, is a promising pharmacological compound for the treatment of alcohol dependence. Specifically, baclofen has been found to suppress symptoms of alcohol withdrawal syndrome with an efficacy comparable to that of diazepam. Moreover baclofen compared to placebo has proven to be effective in the prevention of relapse, likely because of its ability to reduce alcohol intake and craving in alcoholic patients. Baclofen displayed good manageability, as it did not produce any significant side effect. Baclofen appeared to have no abuse liability. Finally, a recent study extended to alcoholic patients affected by liver cirrhosis the efficacy and safety of baclofen in increasing abstinence rate.

The efficacy of the drug in the management of both alcohol withdrawal syndrome and relapse prevention should entail a vastly simplified pharmacotherapy of alcohol dependence.

Symposium 2

PPAR-ALPHA: A POTENTIAL NEW THERAPEUTIC TARGET FOR MOOD DISORDERS

De Montis Graziella[1], Scheggi Simona[1], Pelliccia Teresa[1], Gambarana Carla[1], Melis Miriam[1], Pistis Marco[2]

[1]University of Siena - Siena - Italy, [2]University of Cagliari - Cagliari - Italy

The mesolimbic dopamine (DA) system that has been implicated in reward-related and motivated behaviors plays a major role in adaptive and maladaptive responses.Activity of DA neurons in the VTA is controlled by different afferents and, among these cholinergic inputs play a relevant role through nicotinic acetylcholine receptors (nAChRs) that are targets of peroxisome proliferator-activated receptors-α (PPARα). PPARsα have been shown to modulate β2*nAChRs stimulatory activity on VTA DA neurons upon activation by endogenous endocannabinoids lipids. Consequently PPARα modulation may prove useful in those disorders associated with dysfunction of DAergic transmission, such as depressive states and anhedonia.

To investigate, in well characterized animal models of depressive symptoms, the potential therapeutic activity of the PPARα ligand fenofibrate, and its effect on mesolimbic DAergic transmission we used a combined behavioral, electrophysiological and neurochemical approach in rats.

Rats underwent chronic stress protocols and the potential antidepressant-like activity of fenofibrate was measured in a model of hyporeactivity to aversive stimuli (Escape deficit, ED) and in a model of stress-induced anhedonia. In the latter model the motivation to respond for a rewarding stimulus in a paradigm of SA was studied in control and stressed rats treated or not with fenofibrate. Electrophysiological experiments were carried out in vivo (extracellular single cell recordings in anesthetized animals) from VTA DA neurons of rats repeatedly treated or not with fenofibrate. Immunoblotting examined the effects of repeated fenofibrate on β2*nAChRs phosphorylation in the VTA, and on DA-D1 receptors signaling in terms of DARPP-32 phosphorylation pattern in the NAcS, in stress-exposed and control rats.

a) Repeated fenofibrate administration prevented stress-induced ED and restored a normal reactivity to aversive and pleasurable stimuli in rats exposed to the stress protocol.
b) Repeated fenofibrate treatment reduced β2*-nAChRs phosphorylation levels in the VTA and increased phospho-Thr34-DARPP32 levels in the NAcS in control and chronically stressed rats.
c) The firing pattern of VTA DA cells was modified by the repeated administration of fenofibrate, switching it from a tonic to a bursting state.

Repeated stimulation of PPARα by fenofibrate, possibly by modulation of β2*nAChRs activity, modified mesolimbic DA transmission,reversed the deficit in the acquisition of sucrose SA and the hyporeactivity to noxious stimuli elicited in rats by chronic stress exposure. ED and impairment in the acquisition of an appetitive behavior, i.e. anhedonia, are both expressions of lack of motivation/interest and reproduce distinctive symptoms of major depression. The reinstatement of a normal response to negative and positive stimuli after fenofibrate treatment, strongly suggests an antidepressant-like activity of this compound.

CELLULAR MECHANISMS IN THE LATERAL HABENULA CODING AVERSIVE STATES

Mameli Manuel, Lecca Salvatore

Institut du Fer a Moulin INSERM - Paris - France

Understanding the cellular modifications occurring in the lateral habenula (LHb) upon aversive experience has been proven to be a necessity to gain knowledge on the aetiology of neuropsychiatric disorders. LHb neurons increase their activity upon a punishing aversive experience, and hyperexcitability of LHb neurons is instrumental for depressive like states (Li et al., 2011, Matsumoto and Hikosaka, 2007). Our main hypothesis is that cellular adaptations in the LHb occur early after a negative experience and are determinant for behavioral adaptations, and namely depressive symptoms.

This work aims at understanding how experience linked to aversive states, that include aversive or drug experience, can modify the cellular physiology go lateral habenula neurons,and to causally link these modifications to depressive-like symptoms.

We combined in vitro patch-clamp recordings, pharmacology and behavioral testing in mice to dissect the relationships between cellular modifications and behavioural adaptations.

We find that foot-shock exposure (FsE) drives a reduction in GABAB-GIRK signaling and LHb neuronal hyperexcitability promoting depressive-like behaviors. We find that inhibition of protein phosphatase-2A (PP2A), which regulates the membrane surface of GABAB-GIRK complexes, rescues FsE-driven cellular modifications. As a consequence, PP2A inhibition ameliorates depressive-like phenotypes in rodent models of mood disorders.

These data establish causality between GABAB-GIRK plasticity and LHb hyperexcitability, offering a viable rescue strategy to reverse cellular adaptations and behavioral traits of depression.

THE NEURAL CIRCUIT FOR AVERSION IN CANNABINOID WITHDRAWAL

Muntoni Anna Lisa[1], Aroni Sonia[2], Sagheddu Claudia[2], Pistis Marco[2]

[1]CNR Neuroscience Institute ~ Cagliari - Italy, [2]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari ~ Cagliari ~ Italy

The mesolimbic dopamine (DA) system arising from the ventral tegmental area (VTA) shows a profound reduction in its spontaneous activity after chronic cannabinoid exposure and withdrawal, the critical phases of the drug addiction cycle. These adaptive changes are thought to play a critical role into withdrawal-induced aversive affective states, eventually leading to compulsive drug seeking and relapse. The lateral habenula (LHb) exerts a negative control over the VTA via the GABA rostromedial tegmental nucleus (RMTg), encoding aversion-related stimuli. In fact, both RMTg and LHb cells are activated by negative/unpleasant events, and inhibited by rewarding/positive stimuli. Therefore, these nuclei represent a potential convergence point for drug-evoked reward and aversive opponent processes.

To test the hypothesis that the LHb-RMTg pathway is causally involved in the hypodopaminergic state which occurs during cannabinoid withdrawal.

We used standard single unit extracellular recordings from either VTA, RMTg and LHb neurons in anesthetized male Sprague–Dawley rats. To induce Δ9-tetrahydrocannabinol (Δ9-THC) dependence, rats were chronically treated with Δ9-THC (15 mg/kg, i.p.), or its vehicle, twice daily for 6.5 days. Rats were withdrawn spontaneously or pharmacologically with the cannabinoid antagonist rimonabant (5 mg/kg, i.p.).

Administration of rimonabant precipitated an intense behavioral withdrawal syndrome (one-way ANOVA and Dunnett's test , $p< 0.0001$ versus control), whereas abrupt Δ9-THC suspension caused only milder signs of abstinence (one-way ANOVA and Dunnett's test, $p< 0.01$ versus control). Electrophysiological experiments confirmed that Δ9-THC withdrawal produced a marked decrease in the firing rate and burst firing of VTA DA neurons (one-way ANOVA and Dunnett's test, $p< 0.01$ versus control). As expected, RMTg electrical stimulation elicited a complete suppression of spontaneous activity in approximately half of the DA neurons examined. Remarkably, in Δ9-THC withdrawn rats the duration of RMTg-evoked inhibition lasted longer (one-way ANOVA and Dunnett's test, $p< 0.05$ versus control), suggesting an augmented GABA inhibitory input onto DA cells. By contrast, the spontaneous activity of RMTg GABA neurons was reduced in cannabinoid-withdrawn rats (one-way ANOVA and Dunnett's test, $p< 0.0001$ versus control). Consistent with results, we also found that firing rate of RMTg-projecting LHb neurons was markedly suppressed during cannabinoid withdrawal (one-way ANOVA and Dunnett's test, $p< 0.0001$ versus control).

These findings support the hypothesis that enhanced GABA inputs from RMTg might contribute to the hypodopaminergic state induced by cannabinoid withdrawal, and confirm that the habenulomesencephalic circuitry takes part in the neurobiological mechanisms underlying drug dependence and addiction.

EARLY LIFE STRESS AND RISK FOR PSYCHOPATHOLOGY: A ROLE FOR EPIGENETICS

Luoni Alessia[1], Cattaneo Annamaria[2], Berry Alessandra[3], Cirulli Francesca[3], Riva Marco Andrea[1]

[1]University of Milan ~ Milan ~ Italy, [2]King's College ~ London ~ United Kingdom, [3]Istituto Superiore di Sanità ~ Rome ~ Italy

It is well established that exposure to stressful experiences early in life may determine permanent changes in brain function that enhance the susceptibility to mental illness. With this respect, the use of animal models is instrumental for the identification of the mechanisms that may be responsible for the occurrence of a pathologic phenotype

On these bases, we used the rat prenatal stress (PNS) model to investigate molecular and functional alterations that may contribute to the development or maintenance of the phenotype that originate from the exposure to early life adversity.

We performed target analyses as well as an epigenome-wide study in the prefrontal cortex and hippocampus of male and female rats using a 400K promoter tiling array.

At molecular level, PNS rats show a region- and time-specific reduction in the expression of the neurotrophin BDNF, a marker of neuronal plasticity, which is sustained by the modulation of specific transcripts with the contribution of epigenetic mechanism. Following the epigenome analysis, we found that a large number of gene promoters were differentially methylated in the prefrontal cortex and hippocampus of adult male and female rats exposed to stress during gestation. An overlap of 138 differentially methylated genes around the transcription start site was observed among the two brain regions and genders. Ingenuity Pathway Analysis showed significant enrichment in molecules involved in neurological disease, molecular transport, nervous system development and function as well as psychiatric disorders. By restricting the overlap to genes that were modulated in the same direction, we identified miR-30a as being less methylated in PNS rats. We also employed a convergent cross-species approach to compare the list of genes differentially methylated in PNS rats with methylation changes identified in a cohort of monkeys exposed to maternal separation as well as with changes found in CD34+ stem cells derived from cord blood in human neonates whose mother were grouped on the basis of early life stress exposure. Such analyses allowed us to prioritize the list of genes that are affected by early life adversities and that may therefore play a relevant role for psychopathology and disease susceptibility.

Our data provide support to the notion that early life stress leads to permanent functional and molecular changes in the offspring and highlight the importance of the identification of methylation signatures that may predispose to mental disorders and could ultimately represent a potential target for therapeutic intervention.

Symposium 3

PRION-LIKE PROPERTIES OF C-TERMINALLY TRUNCATED ALPHA-SYNUCLEIN

Bellucci Arianna[1], Navarria Laura[1], Longhena Francesca[1], Pizzi Marina[1], Missale Cristina[1], Spano Pierfranco[2]

[1]Department of Molecular and Translational Medicine, University of Brescia ~ Brescia ~ Italy, [2]IRCCS, San Camillo ~ Venice ~ Italy

A key neuropathological hallmark of Parkinson's disease (PD) is alpha-synuclein deposition in Lewy bodies (LB) in the brain of affected patients. Recently, evidences showing that peripheral alpha-synuclein deposition may precede LB pathology, and that alpha-synuclein may self-propagate and spread progressively in the brain of affected subjects, have conceived the idea that PD may have much in common with prion diseases. This hypothesis is reinforced by the fact that a series of important similarities join alpha-synuclein and prion proteins.

The aim of this study was to investigate whether and how C-terminally truncated alpha-synuclein, a form of the protein that is thought to constitute the core of LB, can be transmitted from cell-to-cell with a cross-species fashion and can activate prion-specific neurodegenerative pathways.

We used a combined biochemical and pharmacological approach to investigate the mechanisms of cell-to-cell transmission of C-terminally truncated alpha-synuclein. In addition, we traced the destiny of the protein after its uptake by recipient cells and evaluated whether, similarly to prion proteins, it activated the unfolded protein response (UPR).

We identified preferential mechanisms of secretion and uptake for C-terminally truncated alpha-synuclein. In addition, we found that this form of the protein can "infect" recipient cells with a prion-like cross-species transmission and then activates specific intracellular pathways that are typical of prion-mediated neurodegeneration.

These findings disclose novel prion-like properties of alpha-synuclein and suggest novel drug targets for therapeutic intervention.

“LYSINES, ACHILLES' HEEL IN ALPHA-SYNUCLEIN CONVERSION TO A NEURONAL OLIGOMERIC ENDOTOXIN “

Plotgher Nicoletta, Berti Giulia, Bisaglia Marco, Greggio Elisa, Bubacco Luigi

University of Padova ~ Padova ~ Italy

Alpha-synuclein (aS) propensity to aggregate is a key element in Parkinson’s disease onset and progression and also in the etiopathogenesis of other disorders associated with the deposition of aS aggregates, generally referred as synucleinopathies. Several toxicity mechanisms have been ascribed to aS oligomerization and aggregation. However, it is still unclear which among the different aggregation pathways is the most relevant to cell toxicity. Conversely, the hypothesis that aS oligomers are the most toxic species to neurons is gaining momentum.
In recent years, mounting evidence suggest that aldehydes accumulation may have a role in aS oligomerization, in line with the observation that several different aldehydes were found to accumulate in parkinsonian brains. The detected molecules were diverse lipid peroxidation products, the dopmine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) and advanced glycation end-products (AGEs).

The aim of our study is the characterization the synergistic toxic effect exerted by DOPAL and aS in the etiopathogenesis and in the spreading of Parkinson’s disease.

The DOPAL dependent aS oligomers were characterized biochemically and structurally by mass spectrometry, NMR and dynamic light scattering. The functional analysis includes a measure of their capacity to permeabilize artificial membranes and an evaluation of their toxicity M17 neuroblastoma cells.

A broad range of biophysical and biochemical techniques were used to characterize the chemical modification of aS due to the reaction with DOPAL and the heterogeneous ensemble of resulting oligomeric aggregated species. aS is modified by DOPAL mainly at lysine residues as independently verified by mass spectrometry and NMR. These DOPAL dependent aS oligomers were then analyzed for their capacity to permeabilize artificial membranes inducing ions leakage. The formation and the toxicity of these aS oligomers have been tested in a cellular model M17 neuroblastoma cells.

The rationale to consider aS more vulnerable than other synaptic proteins to aldehydes covalent modification resides in its intrinsically unfolded nature and in the fact that it has an uncommonly high number of lysine residues compared to any other presynaptic proteins. The aldehydes derived oligomers not only are proposed to be responsible for neuronal toxicity, but they also seem to be efficiently transfer from cell to cell, leading to the propagation of the pathology among neurons.

PUTTING A BRAKE ON AGING TO COUNTERACT PARKINSON'S DISEASE

Decressac Mickael

Telethon Institute of Genetics and Medicine (TIGEM) ~ Pozzuoli ~ Italy

Aging is the primary risk factors of the most common neurodegenerative diseases including Parkinson's disease. In this line, Parkinson's disease can be seen as a stochastic acceleration of dysfunction of cellular pathway governing cellular senescence. Consequently, a better understanding of the molecular pathways underpinning the aging process can lead to the development of novel therapeutic strategies.

Consequently, a better understanding of the molecular pathways underpinning the aging process can lead to the development of novel therapeutic strategies.

Using high-content screening, we have identified an evolutionary-conserved signaling pathway that regulates aging.

We demonstrate that genetic or pharmacological modulation of this pathway provides neuroprotection and regeneration in *in vitro* and *in vivo* models of Parkinson's disease.

Aging manipulation is foremost attractive as a therapeutic approach since it simultaneously targets multiple defensive cellular mechanisms rather than on component of the proteostasis network at a time.

C-REL DEFICIENT MICE, A MOUSE MODEL OF "SPREADING" PD-LIKE PATHOLOGY

Parrella Edoardo[1], Bellucci Arianna[1], Benarese Marina[1], Porrini Vanessa[1], Iorio Rosa[1], Lanzillotta Annamaria[1], Vegezzi Gaia[1], Liou Hsiou Chi[2], Spano Pier Franco[1], Pizzi Marina[1]

[1]Department of Molecular and Translational Medicine, National Institute of Neuroscience, University of Brescia ~ BRESCIA ~ Italy, [2]Department of Immunology Weill Medical College, Cornell University ~ New York ~ United States

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. Together with motor symptomatology, patients affected by PD suffer from non-motor manifestations including olfactory dysfunction and gastrointestinal constipation. Non-motor symptoms precede motor deficits and can play a significant role in the disease-related impairment of quality of life. From a pathological point of view, PD is characterized by the accumulation of alpha-synuclein aggregates, nigrostriatal dopamine (DA) neuron degeneration, synaptic dysfunctions and neuroinflammation. It is now generally accepted that in many PD cases synucleinopathy begins in defined nervous sites and progresses in an anatomically predictable sequence, spreading from periphery (olfactory structures and enteric nervous system) to superior brain regions.
NF-kB factors are considered cardinal players in the progression of the neurodegenerative process. In particular, the c-Rel subunit plays a crucial neuroprotective role, contributing to brain resilience to stress. We have previously shown that mice lacking c-Rel (c-rel-/- mice) develop with aging DA neuronal loss in substantia nigra pars compacta (SNc) with accumulation of aggregated alpha-synuclein, microglia activation and motor deficits responsive to L-DOPA administration.

The characterization of c-rel-/- mice, with particular emphasis on premotor pathology stage. WT and c-rel-/- mice were analyzed at different ages with behavioral tests and biochemical /immunohistochemistry techniques.

We have found that at a premotor pathology stage the c-rel-/- mice suffered from olfactory and gastrointestinal dysfunctions. In parallel with the early non-motor manifestations, young c-rel-/- mice accumulate alpha-synuclein in olfactory bulb and in the enteric nervous system. Moreover, a pathology characterized by alpha-synuclein accumulation in the dorsal nucleus of vagus and locus coeruleus, as well as striatal loss of dopamine transporter, is already present at a premotor pathology stage.

Taken together, these results indicate that c-rel-/- mice may represent an innovative animal model to study pathological progression of PD and to investigate novel therapeutic interventions for early intervention in this disorder.

TRUNCATED HUMAN ALPHA-SYNUCLEIN EXPRESSION RESULTS IN SYNAPTIC DYSFUNCTION AND DOPAMINERGIC CELL DEA

Spillantini Maria Grazia

The Clifford Allbutt Building, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

The pathogenesis of Parkinson's Disease (PD) and other alpha-synucleinopathies is associated with aggregation of alpha-synuclein into oligomers and filaments with the former considered the toxic species. We have previously produced a transgenic mouse expressing 1-120 truncated alpha-synuclein with striatal synaptic alpha-synuclein aggregation and altered dopamine release but no dopaminergic cell death. We have now produced a new transgenic mouse (MI2) expressing higher amounts of the same 1-120 alpha-synuclein transgene under the TH promoter. Here we show that these mice have progressive reduction in dopamine release and dopaminergic cell death in the presence of synaptic alpha-synuclein aggregates. These mice provide an important model to test compounds for the treatment of Parkinson's disease and other alpha-synucleinopathies.

Symposium 4

DRUG REPURPOSING FOR IMMUNE MODULATION IN ISCHEMIC STROKE

Amantea Diana[1], Certo Michelangelo[1], Petrelli Francesco[1], Corasaniti Maria Tiziana[2], Bagetta Giacinto[1]

[1]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria ~ Rende (CS) ~ Italy, [2]Department of Health Sciences, University Magna Graecia of Catanzaro ~ Catanzaro ~ Italy

The majority of the genes acutely modulated in the blood of ischemic stroke patients regulate the immune system and are expressed in circulating neutrophils and macrophages. Accordingly, systemic inflammatory reactions contribute to ischemic stroke injury, prompting activation and brain recruitment of macrophages, neutrophils, dendritic cells and lymphocytes. Cerebral infiltration of blood-borne macrophages and neutrophils exerts a dualistic role on the evolution of brain damage depending on their ability to acquire specific phenotypes. Accordingly, we have recently observed that by reducing cerebral infiltration of neutrophils and detrimental M1 macrophages, while elevating the number of Ym1- and arginase I-immunopositive M2 macrophages in the ischemic hemisphere, the macrolide antibiotic azithromycin provides neuroprotection in a mouse model of transient middle cerebral artery occlusion (MCAo).

Here, we aim at validating whether repurposing existing drugs that target the peripheral immune system, promoting polarization of circulating leukocytes towards non-inflammatory phenotypes, represents a promising strategy for stroke treatment.

Focal brain ischemia was induced by transient (30-min) MCAo with an intraluminal filament in adult C57Bl/6 male mice receiving azithromycin (0.15-150 mg/kg) or bexarotene (0.5-25 mg/kg) i.p. up to 4.5 hours after reperfusion. The peripheral activation and brain infiltration of systemic immune cells was characterised by immunofluorescence labelling of specific cell markers (i.e., CD11b and Iba1 for microglia/macrophages, Ly6B for neutrophils, Ym1 and arginase for M2-macrophages) combined with flow cytometry analysis of brain and peritoneal samples from mice undergone transient MCAo. Arginase activity was assessed in peritoneal exudate cells by measuring the concentration of urea generated by the arginase-dependent hydrolysis of L-arginine.

Neuroprotection afforded by azithromycin is associated to significant elevation of the percentage of F4/80+/Ym1+ M2 macrophages - coincident with increased arginase activity - in peritoneal exudate from mice subjected to transient MCAo. Pharmacological inhibition of peritoneal arginase activity abolishes azithromycin-induced neuroprotection, underscoring the crucial role of drug-induced polarization of migratory macrophages towards a protective, non-inflammatory M2 phenotype. Conversely, by activating PPAR□/RXR receptor, bexarotene promotes peripheral polarization and brain infiltration of N2 neutrophils, thus reducing MCAo-induced brain infarct damage and neurological deficit.

Repurposing existing drugs with immunomodulatory effects represents an innovative strategy for the acute treatment of ischemic stroke.

ROLE OF TRAIL IN FOCAL ISCHEMIA

Di Benedetto Giulia[1], Cantarella Giuseppina[1], Pignataro Giuseppe[2], Di Renzo Gianfranco[2], Annunziato Lucio[2], Bernardini Renato[1]

[1]Department of Biomedical and Biotechnological Sciences, University of Catania ~ Catania ~ Italy, [2]Department of Neuroscience, Division of Pharmacology, School of Medicine, Federico II University of Naples ~ Naples ~ Italy

TNF-related apoptosis inducing ligand (TRAIL), a member of the TNF superfamily released by microglia, appears to be involved in the induction of apoptosis following focal brain ischemia. Indeed, brain ischemia is associated with progressive enlargement of damaged areas and prominent inflammation. It has been demonstrated that the brain's resistance to ischemic injury can be transiently augmented by prior exposure to a non-injurious preconditioning stimulus.

As ischemic preconditioning reduces inflammatory response to brain ischemia and ameliorates brain damage, the purpose of the present study was to evaluate the role of TRAIL and its receptors in stroke and ischemic preconditioning and to propose, by modulating TRAIL pathway, a new therapeutic strategy in stroke.

In order to achieve this aim a rat model of harmful focal ischemia, obtained by subjecting animals to 100 min of transient occlusion of middle cerebral artery followed by 24 h of reperfusion and a rat model of ischemic preconditioning in which the harmful ischemia was preceded by 30 mins of tMCAO, which represents the preconditioning protective stimulus, were used.

Results show that the neuroprotection elicited by ischemic preconditioning occurs through both upregulation of TRAIL decoy receptors and downregulation of TRAIL itself and of its death receptors. As a counterproof, immunoneutralization of TRAIL in tMCAO animals resulted in significant restraint of tissue damage and in a marked functional recovery.

Our data shed new light on the mechanisms that propagate ongoing neuronal damage after ischemia in the adult mammalian brain and provide new molecular targets for therapeutic intervention. Strategies aimed to repress the death-inducing ligands TRAIL, to antagonize the death receptors, or to activate the decoy receptors open new perspectives for the treatment of stroke.

EPIGENETIC DRUGS TO REDUCE POST ISCHEMIC BRAIN INJURY, A NEW THERAPEUTIC APPROACH TARGETING ACETYLATION OF NF-KAPPAB AND HISTONES.

Lanzillotta Annamaria[1], Pignataro Giuseppe[2], Parrella Edoardo[1], Branca Caterina[1], Cuomo Ornella[2], Porrini Vanessa[1], Benarese Marina[1], Annunziato Lucio[2], Spano Pierfranco[1], Pizzi Marina[1]

[1]Dept. of Molecular and Translational Medicine, University of Brescia ~ Brescia ~ Italy, [2]Dept. of Neuroscience, Federico II University of Naples ~ Naples ~ Italy

Nuclear factor-kappaB (NF-κB) p50/RelA plays a dual role in the progression of ischemic stroke. Indeed, it is activated both in protective and lethal events, with a different acetylation profile. In harmful ischemia, but not in a preconditioning insult, neurotoxic activation of p50/RelA is characterized by general deacetylation of RelA, but site-specific acetylation at Lys310 (K310). The derangement of RelA acetylation is associated with reduced histone acetylation and NF-κB-dependent activation of the pro-apoptotic Bim promoter.

In order to correct the altered acetylation of RelA and histones, thus producing neuroprotecion, we evaluated the combination of the clinically used histone deacetylase (HDAC) inhibitors, entinostat (MS-275) or valproic acid (VPA), in combination with the sirtuin-1 activator resveratrol. We used both in vitro and in vivo models of brain ischemia: oxygen glucose deprivation (OGD) in primary cortical neurons and the transient middle cerebral artery occlusion (MCAO) in mice.

In primary neurons exposed to OGD the combined use of resveratrol and a HDAC inhibitors, MS-275 or VPA, led to a synergistic neuroprotection. Indeed, in neurons co-exposed to MS-275 and resveratrol the abnormal acetylation of RelA is reverted through an increase of general acetylation, mediated by MS-275, and a specific decrease of K310 acetylation, mediated by resveratrol through an AMP-activated protein kinase/sirtuin 1 pathway. Moreover, the optimal histone H3 acetylation was recovered in neurons treated with the synergic combination of drugs.
The treatment with MS-275 (20 µg/kg and 200 µg/kg), resveratrol (6800 µg/kg) or VPA (20 mg/kg) by themselves in mice exposed to MCAO elicited neuroprotection. However, the combination of MS-275 or VPA with resveratrol at 100-fold lower concentrations induced a significant reduction in both infarct volume and neurological deficits, even when administered 7 hours after the stroke onset. Additionally, chromatin immunoprecipitation analysis in cerebral cortices harvested from mice exposed to MCAO and treated with MS-275 and resveratrol or vehicle showed that the treatment induced the shifiting of RelA binding and histone acetylation from the Bim to the Bcl-xL promoter.

Epigenetic therapy targeting RelA and histone acetylation may be a promising strategy to limit post-ischemic injury with an extended therapeutic window.

IONIC HOMEOSTASIS AND ENDOGENOUS NEUROPROTECTION

Pignataro Giuseppe, Cuomo Ornella, Vinciguerra Antonio, Sirabella Rossana, Di Renzo Gianfranco, Annunziato Lucio

Dept. Neuroscience, School of Medicine, Federico II University of Naples ~ Naples ~ Italy

Most of the current focus on developing neuroprotective therapies is aimed at preventing neuronal death. Recently, the research in this field aimed to develop strategies which induce, mimic, or boost endogenous protective responses. Using a mild insult to induce endogenous neuroprotective mechanisms is known as preconditioning. When followed by a severe insult, or challenge, preconditioning results in a state of tolerance in which the injury inflicted by the challenge is mitigated. A new promising approach for neuroprotection derives from postconditioning, in which neuroprotection is achieved by a modified reperfusion subsequent to a prolonged ischemic episode.

Although mechanisms through which these two endogenous protective strategies exert their effects are not yet fully understood, robust evidence highlight the importance of proteins deputed to the ionic homeostasis maintenance. The main aim of this work was to unravel the role in brain conditioning neuroprotection of two proteins involved in stroke pathophysiology: the plasmamembrane transporters Na+/Ca2+ exchangers (NCXs), and the acid-sensing cation channels (ASIC).

NCX and ASIC have been characterized in terms of expression, transcriptional regulation and role in mediating conditioning-induced protection in brain of ischemic, preconditioned and postconditioned rats. Experimental stroke has been induced by 100 minutes of transient occlusion of the Middle Cerebral Artery in adult male rats. In the preconditioning protocol a subliminal 30 minutes occlusion preceded the harmful stroke, whereas in the postconditioning model a 10 minute occlusion was induced 10 minutes after hrmful stroke.

NCX and ASIC are differently regulated by the endogenous neuroprotective strategies termed preconditioning and postconditioning. In particular, brain conditioning-mediated neuroprotection occurs through NCX upregulation and ASIC1a downregulation. These effects are mediated through AKT and are influenced by the hypoxic-inducible factor, HIF. More interestingly, the expression of NCX is tightly controlled by the family of microRNA named miRNA 103-107 whose expression is regulated either locally and peripherally.

NCX and ASIC emerge as pivotal targets in setting on new effective stroke interventions.

Symposium 5

SHANK3 INSUFFICIENCY UNCOVERS A ROLE OF VTA IN ASDS

Bellone Camilla

University of Lausanne, DNF ~ Lausanne ~ Switzerland

Autism spectrum disorders are a group of highly heritable diseases characterized by deficits in social behaviors. Loss of a functional copy of SHANK3 or mutations in SHANK3 gene is the most common monogenic cause of ASDs. Shank3 is a scaffolding protein enriched at the postsynaptic density of excitatory synapses where via its different domains orchestrates metabotropic and ionotropic receptors at glutamatergic synapses.

Although many studies underlie the importance of Shank3 deletions in defining synaptic deficits and behavioral phenotypes, which are the circuits that code for social impairments and whether synaptic deficits are directly accountable for the behavioral outcomes are still open questions that need to be addressed if we want to find new therapeutic targets.

We use In vitro and in vivo recordings together with behavioural approaches

Here we show that mice carrying a Shank3 insufficiency in the VTA during the postnatal development exhibit an augmented AMPA/NMDA ratio together with an increased expression of GluA2-lacking AMPARs. These synaptic alterations do not occur when Shank3 is dowregulated at adolescent synapses suggesting a deficit in synaptic maturation at excitatory inputs onto DA neurons. Importantly the insufficiency of Shank3 in the VTA leads to a reduction in social preference during the social interaction task, indicating a decrease in social motivation. Finally we show that treatment of the mice with a positive allosteric modulator of metabotropic glutamate receptor 1 during the critical period of development restores AMPAR transmission and social deficits throughout adulthood.

These results altogether suggest that impaired postnatal development of glutamatergic transmission onto DA neurons of the VTA contributes to the development of social deficits in Shankopathies and that mGluR1 modulation offer a potential strategy to treat ASDs.

SHORT-TERM ABSTINENCE FROM DEVELOPMENTAL EXPOSURE TO COCAINE ALTERS THE GLUTAMATE SYNAPSE FOLLOWING ACUTE STRESS: IMPLICATION OF GLUCOCORTICOID RECEPTORS.

Fumagalli Fabio, Caffino Lucia, Giannotti Giuseppe, Racagni Giorgio

University of Milan ~ Milan ~ Italy

Adolescence is a developmental period characterized by impulsive choices that may lead to the beginning and escalation of illicit drug use. In this stage of life, the brain is in a unique state of transition as it undergoes profound structural and synaptic changes and, therefore, interfering with brain development during this delicate period may cause adverse consequences. Such effects may be the result of long-term neuroadaptations that involve, among the others, the glutamate system.

The main aim of our study was, therefore, to evaluate the response of the glutamate system to the long-term exposure to cocaine (20mg/kg/day) during adolescence [from postnatal day (PND) 28 to PND 42] and whether the rapid coping response of the glutamatergic synapse to an acute stress (5 minutes of swim stress and killing 15 min later) was influenced by the previous cocaine history.

Critical determinants of glutamatergic homeostasis were measured in the medial prefrontal cortex (mPFC), by means of Real Time PCR and Western blots. Circulating corticosterone levels were analyzed by ELISA and the time of immobility was measured by three independent investigators blind to the experimental design. Morphological analyses on dendritic spines were taken using a fluorescent dyolistic labeling technique.

The developmental exposure to cocaine influenced the response of the glutamatergic synapse to the acute stress by increasing the vesicular glutamate transporter, reducing glial glutamate transporters and increasing the activation of the NMDA receptor. This results in the activation of Cdc42 and phosphoPAK1 that may cause changes in spine structural plasticity. Notably, these effects were independent from the circulating levels of corticosterone. Additionally, cocaine-withdrawn rats exposed to stress spent more time immobile then their saline counterparts suggesting a pro-depressive phenotype. We also found increased GR transcription and translation as well as increased nuclear translocation of GR, associated with the reduced expression of the GR co-chaperone FKBP5 that, under physiological conditions, keeps the receptor in the cytoplasm, in the mPFC of cocaine-exposed PND 45 animals.

These results indicate a coordinated series of changes, presumably through cocaine-induced reduction of baseline mPFC neuronal activity. This may result in a hyper-reactive glutamatergic synapse in the mPFC of rats with a prior cocaine history, when exposed to an acute stress. This may occur, at least in part, via changes in stress-related mechanisms that may contribute to the depressive-like behavior observed in early cocaine withdrawal.

PRENATAL EXPOSURE TO CANNABINOIDS AFFECTS POSTNATAL SYNAPTIC PROPERTIES AND PLASTICITY OF EXCITATORY TRANSMISSION ON DOPAMINE NEURONS OF THE VENTRAL TEGMENTAL AREA.

Aroni Sonia, Sagheddu Claudia, Melis Miriam

Dept. Biomedical Sciences ~ Cagliari ~ Italy

Early life adverse events can persistently affect brain functions, and contribute to the development of psychiatric trajectories. Hence, exposure to drugs of abuse early in life produces long-lasting cognitive deficits in humans. Particularly, prenatal Cannabis exposure has negative impact in humans on cognitive processing, such as reduced attention processing and impairments in motor and language skills as well as associative and discrimination learning. Despite the high prevalence of Cannabis use among pregnant women, its impact on the developing brain is still not accurate.

The present study was aimed at investigating on the effects of prenatal cannabinoid exposure on i) the postnatal maturation of glutamatergic transmission onto ventral tegmental area (VTA) dopamine (DA) neurons in the offspring; ii) endocannabinoid-mediated short-term plasticity at excitatory synapses onto VTA DA cells.

To this aim, Sprague Dawley dams were administered the psychoactive ingredient of Cannabis, 9-tethrayhydrocannabinol (THC, 2 mg/kg s.c.), once per day from GD 5 to GD 20.

We found that the off-spring of cannabinoid-exposed dams, during the third postnatal week, displayed a glutamatergic transmission dominated by calcium-permeable AMPA receptors. The delayed AMPA receptor switch in THC-offspring was accompanied by a paired-pulse facilitation and an increased AMPA to NMDA ratio. In addition, changes in endocannabinoid-mediated short term plasticity at these synapses were observed, which appear not to be related to CB1 receptor number/function.

The present data suggest that maternal Cannabis use alters developmental regulation of mesolimbic DA system, which might result in enduring changes in brain function and abnormal behavior. Whether or not these changes are long lasting and/or might contribute to affective dysregulation and addiction vulnerability later in life has to be examined yet.

THE IMPACT OF ADOLESCENT CANNABINOID EXPOSURE ON BRAIN MATURATION: FOCUS ON EPIGENETIC MECHANISMS

Prini Pamela, Penna Federica, Zamberletti Erica, Gabaglio Marina, Parolaro Daniela, Rubino Tiziana

DiSTA University of Insubria ~ Busto Arsizio ~ Italy

Heavy adolescent exposure to THC in female rats disrupts the physiological maturation of the endocannabinoid system and negatively impacts developmental changes typical of the adolescent brain, thus leading to altered adult brain functionality and behavior. However, the molecular underpinnings that make the adolescent brain so vulnerable to THC adverse effects are not yet fully understood. The emerging role of epigenetic mechanisms in the development of psychiatric diseases led us to hypothesize that alterations in epigenetic modifications could play a part in THC-induced modification of adolescent developmental changes

The main goal of this work was to investigate whether epigenetic mechanisms may contribute to the events triggered by adolescent THC exposure.

To this aim, we first performed a time-course study of different histone H3 modifications occurring in the prefrontal cortex (PFC) of female rats exposed to THC during adolescence. We next investigated how the epigenetic alterations induced by THC are translated into transcriptional changes, focusing our attention on a subset of genes closely related to the ECS and neuroplasticity processes. Moreover, to verify the vulnerability of the adolescent brain, we performed the same analysis after adult THC exposure. Finally, through the administration of a specific epigenetic drug, we investigated the role played by histone modifications in the complex phenotype present in adult animals after adolescent THC exposure

Adolescent THC exposure induced alterations in selective histone modifications (mainly H3K9me3) that impacted the expression of a set of genes closely related to synaptic plasticity mechanisms. Changes in both histone modifications and gene expression were more widespread and intense after adolescent treatment in comparison with that of adults, suggesting the existence of adolescent vulnerability. Moreover, adolescent THC exposure significantly increased protein expression of the histone methyl transferase Suv39H1 that could account for the enhanced H3K9me3. This modification played a relevant role in the development of the depressive/psychotic-like phenotype induced by adolescent THC exposure. Indeed, pharmacological blockade of Suv39H1 during adolescent THC treatment was able to prevent THC-induced cognitive deficits at adulthood

Together these results suggest that in the adolescent prefrontal cortex, THC acts through Suv39H1 to affect histone modifications and gene expression. This pathway appears to be relevant for the development of cognitive deficits present at adulthood.

Symposium 6

NGF AND GLIAL CELLS: AN OVERLOOKED STORY

Capsoni Simona

Scuola Normale Superiore ~ Pisa ~ Italy

Over the last decade, a series of studies has demonstrated that glia (astrocytes and microglia, in particular) in the central nervous system play roles in many aspects of neuronal functioning including chronic pain processing. Peripheral tissue damage or inflammation initiates signals that alter the function of the glial cells , which in turn release factors that regulate nociceptive neuronal excitability. The interactions between astrocytes, microglia and neurons are now recognized as fundamental mechanisms underlying acute and chronic pain states. On the other hand, the neurotrophin Nerve Growth Factor (NGF) plays a major role in the pathophysiology of chronic, inflammatory pain. In fact, sensory neurons, called nociceptors, require NGF for their development and functional maturation. However, the role of NGF in chronic pain might not be restricted to the functional regulation of sensory neurons that signal the first events that lead to pain, but it might also influence glial activity.

For a long time it has been suggested (but also overlooked) that the expression of NGF and its receptors TrkA and p75NTR might be upregulated in microglia and astrocytes during pathological states. In this review, I will focus on new data on the action of NGF on glial cells.

Starting from the phenotypic analysis of a mouse model of NGF deprivation, we applied morphometric, biochemical and gene expression analysis to test the influence of NGF on astrocytes and microglia activity.

We found that NGF deprivation provokes robust changes in glial morphology, astrocyte calcium oscillation and microglial phagocytic activity.

These data pave the way for further understanding of chronic pain mechanisms and future development of effective treatments.

TRPV1 CHANNELS IN THE BRAIN: MODULATION OF MICROGLIA TO NEURON COMMUNICATION IN NAIVE AND NEUROPATHIC MICE

Marrone Maria Cristina[1], Morabito Annunziato[1], Giustizieri Michela[1], Marinelli Sara[2], Riganti Loredana[3], Murana Emanuele[7], Maccarrone Mauro[4], Oddi Sergio[5], Ragozzino Davide[7], Verderio Claudia[6], Marinelli Silvia[1]

[1]Fondazione EBRI Rita Levi Montalcini ~ Roma ~ Italy, [2]CNR National Research Council of Italy, Cell Biology and Neurobiology Institute ~ Rome ~ Italy, [3]Department of Medical Biotechnology and Translational Medicine, University of Milano ~ Milan ~ Italy, [4]Campus Bio-Medico University of Rome ~ Rome ~ Iceland, [5]Faculty of Veterinary Medicine, University of Teramo ~ Teramo ~ Italy, [6]CNR Institute of Neuroscience; IRCCS Humanitas ~ Milan ~ Italy, [7]Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Physiology and Pharmacology, Sapienza University of Rome ~ Rome ~ Italy

The capsaicin receptor TRPV1 belongs to the broad TRP channel family that senses both the external stimuli and alterations of the intracellular and extracellular milieu. TRPV1 has been highly characterized in the sensory system especially as key component of pain and inflammation. Although at lower level, this channel is also functionally present in the brain. When expressed in the postsynaptic compartment TRPV1 activation modulates synaptic plasticity decreasing the synaptic strength, whereas facilitates neurotransmission by a presynaptic mechanism. However, TRPV1 expression in the brain is still under debate because of its highly restricted expression in the CNS and the nature of its endogenous activation such as thermal stimuli >42-43°C.

Deeper investigation on both expression and functional role of TRPV1 in brain areas, i.e the anterior cingulate cortex (ACC).

To address these aims, we used adult naïve, sham and mice suffering from neuropathic pain (NP). As controls, TRPV1KO mice were also employed. Murine model of NP: chronic constriction injury of the sciatic nerve (CCI). Ex-vivo patch-clamp recordings of ACC layer 2-3 pyramidal neurons and immunofluorescence assays from the above mice.

We found that TRPV1 is mainly expressed in microglial cells rather than neurons in the cortex and other brain areas of control mice. TRPV1 activation by capsaicin shifts microglia from ramified to hypertrophic shape, stimulates the shedding of extracellular vesicles and induces an outwardly rectifying current in microglial cells. Furthermore capsaicin tunes glutamatergic neurotransmission by triggering the release of microglial factors that interact with their downstream receptors, i.e. the purinergic, metabotropic glutamatergic and tyrosine kinase A receptors. Importantly, in the cortex of mice suffering from NP, TRPV1 is also present in neurons where it regulates both their intrinsic electrical properties and synaptic strength by a postsynaptic mechanism.

These findings identify a new role for microglial TRPV1 as potential detector of harmful stimuli and key player in microglia to neuron communication in the brain.

SORTILIN – A NEW TARGET FOR TREATMENT OF NEUROPATHIC PAIN?

Richner Mette, Vaegter Christian, Nykjaer Anders

Aarhus University ~ Aarhus ~ Denmark

Tactile allodynia, the painful response to normally innocuous stimuli, may result from injury to the peripheral nervous system. Despite its debilitating nature the underlying molecular mechanisms remain poorly understood precluding development of targeted drugs. A number of signaling molecules including neurotensin, substance P, and CCK have been implicated but best described is the down-regulation of the chloride-transporter KCC2 by BDNF and TrkB leading to attenuated GABA signaling and sensory disinhibition.

Sortilin, a member of the Vps10p-domain receptor family and expressed in dorsal horn of the spinal cord is a high affinity receptor for neurotensin and a regulator of TrkB signaling. We wish to investigate a possible role for sortilin in neuropathic pain.

Using biochemical methods and the spared nerve injury followed by Von Frey test we have studied tactile allodynia in sortilin knockout mice. Furthermore, we characterize the first small molecule sortilin receptor antagonist and study its relevance to neuropathic pain

We find that sortilin deficient mice are protected against neuropathic pain by preventing down regulation of KCC2. Further, we identify the ligand that accounts for this effect.

Our findings identify sortilin as a novel drug target for treatment of neuropathic pain.

Symposium 7

IMPLICATIONS OF FAMILIAL MUTATIONS FOR THE PATHOGENESIS AND TREATMENT OF NEURODEGENERATIVE DISEASES

Harvey Kirsten

University College London ~ London ~ United Kingdom

Genetic forms of neurodegenerative diseases such as familial Alzheimer's (AD) and Parkinson's disease (PD) allow for the investigation of the physiological and pathogenetic function of the affected genes. Discovering the function of these genes and determining how dysfunction can cause changes in cellular function is likely to be a crucial step on the journey towards finding a cure for these devastating diseases. Mutations in LRRK2 are a major cause of PD. Changes in LRRK2 activity were shown to cause alterations in Wnt signalling. Deregulated Wnt signalling has been increasingly linked to neurodegenerative disease including AD. Cell biological functions disrupted in PD are partially controlled by Wnt signalling cascades and proteins encoded by the PARK genes PARKIN, LRRK2, VPS35 and PINK1 have been shown to modify Wnt signalling. This suggests the prospect of targeting Wnt signalling pathways to slow or halt the progression of PD.

To investigate the role of wild-type and mutant LRRK2 in the regulation and deregulation of canonical Wnt signalling.

Use of LRRK2 knockout and G2019S knock-in models to conduct functional Wnt signalling assays. Immunocytochemistry, in vivo imaging, co-immunoprecepitation and pull down in mouse brain and cell models.

Our results indicate that LRRK2 functions as a scaffold protein in membrane and cytoplasmic Wnt signalling complexes. Experiments performed in LRRK2 wild-type and knockout models demonstrate that LRRK2 functions as a β-catenin destruction complex component and that loss of LRRK2 stimulates Wnt activity. We further present evidence that LRRK2 mutations and risk variants weaken canonical Wnt activity whereas protective variants increase Wnt signal activation. Thus our data implicate LRRK2 as a central scaffold protein in canonical Wnt signalling, and suggest that LRRK2 mutations contribute to the pathogenesis of PD by repressing this crucial signal transduction pathway.

We conclude that LRRK2 functions as a key Wnt signalling component. Our data reveal a clear correlation between canonical Wnt signalling activity and the risk of and protection from developing PD. Familial PD mutations decrease Wnt signalling activity whereas protective variants increase the activity of this pathway. Decrease in Wnt signalling activity has previously been linked to neurodegeneration and provides therefore a plausible mechanism and a good therapeutic target for a disease modifying treatment of PD. Wnt signalling pathway components are easily targeted with e.g. small molecules. Therefore, our research not only has relevance for basic biology and the pathogenesis of neurodegeneration, but also for new therapeutic interventions.

STEM CELL-BASED MODULATION OF INNATE AND ADAPTIVE IMMUNE RESPONSES IN CNS INJURY

Peruzzotti--Jametti Luca[1], Mallucci Giulia[1], Joshua Bernstock[1], Volpe Giulio[1], Bicci Iacopo[1], Balzarotti Beatrice[1], Donegà Matteo[1], Henriques Da Costa Sofia[2], Sciacovelli Marco[2], Gaude Edoardo[2], Leonardi Tommaso[1], Iraci Nunzio[1], Giusto Elena[1], Tannahill Gillian[1], Frezza Christian[2], Edenhofer Frank[3], Pluchino Stefano[1]

[1]University of Cambridge, Department of Clinical Neurosciences ~ Cambridge ~ United Kingdom, [2]MRC Cancer Unit, University of Cambridge ~ Cambridge ~ United Kingdom, [3]University of Würzburg, Institute for Anatomy and Cell Biology ~ Würzburg ~ Germany

Evidence has emerged to indicate that transplantation of neural stem/precursor cells (NPCs) promotes the recovery of the injured central nervous system (CNS) via the modulation of the host's immune system. Sustained stem cell graft-to-host communication leads to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on both the innate and adaptive immune responses.

Herein, we focus on the main cellular signalling pathways that grafted NPCs use to establish a therapeutically relevant cross talk with host immune cells, while examining the role of inflammation in regulating some of the bidirectionality of these communications.

We show that the transplantation of induced neural stem cells (iNSCs), a novel autologous neural stem cell source obtained directly from somatic fibroblasts via cellular reprogramming, is capable of modifying the innate inflammatory response of experimental autoimmune encephalomyelitis (EAE) mice.

Upon transplantation, iNSCs accumulate/persist in close proximity to mononuclear phagocytes (MPs), thereby inducing a switch of invading MPs from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype. Interestingly, in vitro evidence suggests that iNSCs (as well as NPCs) are capable of influencing the innate immune response via a novel mechanism of metabolic reprogramming .

We propose that the identification of the players involved in stem cell signaling might contribute to the development of innovative, high clinical impact therapeutics for inflammatory CNS diseases. In particular, metabolic changes underpinning macrophages polarization could be specifically targeted for the treatment of CNS disorders.

NEUROINFLAMMATORY CYTOKINES AND THEIR RECEPTORS AS INNOVATIVE TARGETS: RESTRAINT OF NEURONAL DAMAGE AND RESTORATION OF FUNCTION IN AD

Cantarella Giuseppina, Di Benedetto Giulia, Puzzo Daniela, Loreto Carla, Saccone Salvatore, Bernardini Renato

University of Catania ~ Catania ~ Italy

Alzheimer disease (AD) is one of the most common causes of death worldwide, with poor treatment options. A tissue landmark of AD is accumulation of beta-amyloid (Aβ) in specific brain areas. Nevertheless, AD pathogenesis is thought to be substantially contributed by different factors, including inflammation that occurs concomitantly with deposition of Aβ in the brain tissue, and is associated with both gliosis and release of various mediators of the inflammatory/immune response. We have previously shown that TNFSF-10 a is released by human neural cells challenged with Aβ n vitro, and, in addition, by activated glia. Interestingly, neutralization of TRAIL is associated with rescue from death of neurons challenged with Aβ in vitro as well as with significant functional recovery in animal models of nervous tissue injury in vivo.

Based on these findings, we verified whether TNFSF-10 could play a pathophysiological role in AD, and whether its neutralization might bring about improvement of cognitive impairment. To do so, the expression of TNFSF-10 and its receptors, as well as cognitive parameters, were studied in the triple transgenic mouse model of AD (3xTg-AD). To corroborate the above hypothesis of a role of TNFSF-10 in the cognitive impairment of 3xTg-AD mice, the effects of chronic anti- TNFSF-10 treatment on inflammation and cognitive function were next evaluated in these animals.

Mice were treated i.p. twice a month for 6 months with a TNFSF-10 neutralizing Mab. The expression of TNFSF-10, DR5 Ab COX2, GFAP, IL-1b and NOS2 were studied by western blot and by immunohistochemistry. Immunofluorescence studies were performed for brain localization of NOS2, GFAP and TNFSF-10-neutralizing antibody. Morris water maze and the novel object recognition tests were performed for behavioural studies. Data were expressed as mean ± standard error mean (SEM). For behavioural studies, statistical analysis was performed by using a software.

We demonstrate that blocking TNFSF-10 by administration of a neutralizing monoclonal antibody could attenuate the Aβ-induced neurotoxicity in a 3xTg-AD mice. Treatment with the TNFSF-10 neutralizing antibody resulted in dramatic improvement of cognitive parameters and it correlated with decreased protein expression of TNFSF-10, Aβ, inflammatory mediators and GFAP in the hippocampus.

We demonstrated that neutralization of TNFSF-10 results in dramatic functional recovery in models of severely impaired cognitive behaviour. It appears plausible to hypothesize that TNFSF-10 is a pivot mediator of neuronal damage consequent to Aβ-related neuroinflammation. Targeting the TNFSF-10 machinery with immunopharmacological modalities could be envisioned as a potential beneficial treatment for amyloid-related neurodegenerative disorders.

TARGETING WNT SIGNALING FOR DOPAMINERGIC (DA) NEUROREPAIR IN PARKINSON'S DISEASE: TRIPARTITE DIALOGUE BETWEEN DA NEUROPROGENITORS, GLIAL CELLS AND THE INJURED DA NEURONS.

Marchetti Bianca[1], L''Episcopo Francesca[2], Tirolo Cataldo[2], Testa Nunzio[2], Serapide Maria Francesca[3]

[1]Dept. of Biomed and Biotechnol Sci. Pharmacol. Sections, University of Catania Medical School ~ Catania ~ Italy, [2]OASI Sci. Inst. for Res. and Care (IRCCS), Neuropharmacol Section ~ Troina (EN) ~ Italy, [3]Dept. of Biomed and Biotechnol Sci.Physiol.Sections ~ Catania ~ Italy

Parkinson's disease (PD) is a chronic neurodegenerative disorder, characterized by a progressive loss of midbrain dopaminergic (mDA) neuronal cell bodies in the subtantia nigra pars compacta (SNpc) and their terminals in the striatum, resulting in a debilitating disorder with dysfunctions of the motor system. Currently, available pharmacological and surgical therapies ameliorate clinical symptoms in the early stages of disease, but they cannot stop or reverse degeneration of mDA neurons. Remarkably, adult mDA neurons are endowed with a panel of endogenous self-repair mechanisms and neuro-immune networks regulating neuroprotective and regenerative processes in the injuried SNpc. However, most of these self-defense mechanisms are impaired with the aging process and according to specific gene-environment interactions.

However, most of these self-defense mechanisms are impaired with the aging process and according to specific gene-environment interactions. Thus, compensation for the progressive loss of mDA neurons during PD using current pharmacological treatment strategies is limited and remains challenging. Given the chief role of Wingless-type MMTV integration site (Wnt) signaling cascade as an essential regulator of development and maintenance of mDAergic neurons, and involved in neuroimmune-modulation, we herein address the possibility to boost endogenous self-repair mechanisms in nigrostriatal DA degeneration by underlying the role of Wnt signaling in the interplay between glial cells, DA neuroprogenitors, and the injured mDA neurons for nigrostriatal restoration/repair.

We highlight an intrinsic Wnt/neuroinflammatory response triggered upon SNpc injuries and the many ways by which Wnt operates, forming an integral program for SNpc self-renewal and regeneration.

Our current work convincingly demonstrated that the immune system is intimately tied to mechanisms of adult neurogenesis, and that Wnt signaling cascade is a critical intermediate. Key actors of this scenario are astrocytes, providing neuroprotective effects against DA degeneration and playing fundamental roles in DA differentiation of neural stem cells. Moreover, together with macrophage/microglial cells, astrocytes shape the niche microenvironment, down-regulating excessive inflammation and activating the transcription of morphogens and master anti-oxidant regulators, to engage stem/neuroprogenitor cell (NPC) activation, neuroprotection and DA neuron replacement/repair through intricate tripartite interactions.

The plasticity of this sytem and the potential to target endogenous neurorepair with cell/pharmacological approaches for therapeutic intervention in PD is discussed.

Symposium 8

THE "SPATIAL AND QUANTITATIVE CODE" OF BDNF VARIANTS: A THERAPEUTIC TARGET FOR THE DISORDERS OF THE NERVOUS SYSTEM

Baj Gabriele, Polacchini Alessio, Pinheiro Vera, Vicario Annalisa, Vaghi Valentina, Tongiorgi Enrico

University of Trieste, Life Sciences Dpt. ~ Trieste ~ Italy

The neurotrophin Brain-Derived Neurotrophic Factor (BDNF) is a key molecule in neuronal development and plasticity, and one of the major pharmaceutical targets in neuropsychiatric diseases and neurodevelopmental disorders. Any effort to modulate pharmacologically this neurotrophin is complex, since BDNF is produced by several spliced transcripts, which result from 11 different 5' untranslated regions (UTRs). These 5'UTRs are linked to a common coding region with two alternative 3'UTRs. The diverse BDNF transcripts are differentially distributed in three subcellular regions: the soma, the proximal and the distal dendrites, thus acting as a spatial code for mRNA localization.

Since, the signaling pathways governing BDNF translation in distal, high-order dendritic branches remain unclear, the objectives of our research is to understand the regulation of BDNF translation in dendrites.

We analyzed the translatability of exons coding for the different BDNF 5'UTRs (11) and 3' UTR tails (2) using an in vitro Luciferase assay developed in our lab (Patent PCT/EP2010/067081). In addition, we preformed immunofluorescence staining of BDNF protein and translational machinery elements in rat hippocampal neurons. We used a vector encoding for exon6-BDNFcds-3'UTR in fusion with GFP to study mRNA (FISH) and protein localization (IF) upon neuronal activation.

We quantified the different translatability of each 5' and 3' UTR BDNF sequences in basal condition and in response to a panel of agonists. Moreover, we identified the signaling cascades controlling activity-dependent BDNF local protein translation in hippocampal neuronal dendrites. Finally, we described the mRNA trafficking and the translational regulation of BDNF exon 6 transcript, which is one of the most abundant BDNF mRNA variant in dendrites.

We concluded that the different BDNF transcripts act as a quantitative code regulating BDNF protein levels. The principal components of the signalling pathways involved in BDNF translation were found to be constitutively distributed along the dendrites, while others were strongly regulated by neuronal activity. The determination of signalling pathways controlling the exon-6-CDS-3'UTR-long transcript translation showed that the mRNA localization acts as spatial code for specific BDNF production. These results may contribute to develop more specific drugs for neuronal diseases in which BDNF plays an important role.

SILENCING BDNF EXPRESSION WITH HERPES SIMPLEX VIRUS TYPE-1 BASED AMPLICON VECTORS IN AN EXPERIMENTAL MODEL OF TEMPORAL LOBE EPILEPSY

Falcicchia Chiara[1], Trempat Pascal[2], Binaschi Anna[1], Soukupova Marie[1], Roncon Paolo[1], Berthommé Hervé[2], Simonato Michele[1]

[1]Department of Medical Science, Section of Pharmacology, Neuroscience Center, University of Ferrara and National Institute of Neuroscience ~ Ferrara ~ Italy, [2]Bioviron, Univ. Claude Bernard-Lyon I ~ Villeurbanne ~ France

Brain-derived neurotrophic factor (BDNF) has been found to produce pro- but also anti-epileptic effects. Thus, its validity as a therapeutic target must be verified using advanced tools designed to block or to enhance the BDNF signal.

The aim of this study was to develop tools to silence the BDNF signal. We generated Herpes simplex virus type 1 (HSV-1) derived amplicon vectors, i.e. viral particles containing a genome of 152 kb constituted of concatameric repetitions of an expression cassette, enabling the expression of the gene of interest in multiple copies.

HSV-1 based amplicon vectors are non-pathogenic and have been successfully employed in the past for gene delivery into the brain of living animals. Amplicon vectors are thus a relevant and reliable choice for expressing a silencing cassette, which, in multiple copies, is expected to lead to an efficient knock-down of the target gene expression. Two BDNF silencing strategies have been developed. The first, antisense, has been chosen to target and degrade the cytoplasmic mRNA pool of BDNF, whereas the second, based on the convergent transcription technology, has been chosen to repress transcription at the BDNF gene.

Both these amplicon vectors proved to be effective in down-regulating BDNF expression in vitro, in BDNF-expressing mesoangioblasts cells. However, only the antisense strategy was effective in vivo, after inoculation in the hippocampus in the pilocarpine model of temporal lobe epilepsy, in which BDNF mRNA levels are strongly increased. Interestingly, the knock down of BDNF levels induced with BDNF-antisense was sufficient to produce significant behavioral effects, in spite of the fact that it was produced in a part of a single hippocampus and not in the entire epileptogenic area.

In conclusion, this study demonstrates a reliable effect of amplicon vectors in knocking down gene expression in vitro and in vivo. Therefore, this approach may find broad applications in neurobiological studies.

ARC EXPRESSION IS MODULATED POST-TRANSCRIPTIONALLY BY SPLICING OF ITS 3'UTR AND BDNF SIGNALLING

Ricciardi Simona[1], Okenwa Chinenye[1], Catalanotto Caterina[2], Ciotti Maria Teresa[1], Cattaneo Antonino[1], Cogoni Carlo[2], Giorgi Corinna[1]

[1]EBRI ~ Rome ~ Italy, [2]Univ. of Rome "Sapienza", Department BCE ~ Rome ~ Italy

Arc protein is a key player in synaptic homeostasis, plasticity and memory consolidation, regulating surface AMPA-Rs abundance in response to synaptic activity. As an immediate early gene, Arc mRNA is strongly induced by synaptic activation and a portion is actively transported and locally translated in dendrites where it elicits AMPA-Rs endocytosis at un-stimulated synapses. As a key regulator of homeostatic balance, Arc expression is highly regulated and transient. Several mechanisms are devoted to restrain Arc abundance, including a tight post-transcriptional regulation: upon activation, a portion of its newly transcribed mRNA pool is translationally silenced (via interaction with FMRP and miRNAs), transported to dendrites, locally translated and rapidly degraded both at the mRNA and protein levels. Cis-acting elements that control these steps post-transcriptionally are encoded primarily in Arc mRNA 3'untranslated region (3'UTR), including two dendritic targeting sequences, an element likely mediating FMRP translational repression, and several miRNA binding sites. Additionally, as shown previously, Arc 3'UTR contains two conserved introns, which uniquely modulate Arc mRNA stability by targeting it for destruction by the Nonsense Mediated Decay pathway (NMD).

Aim of this study is to further characterize how Arc mRNA 3'UTR region contributes to the modulation of Arc expression in neurons.

To this end, mammalian expression plasmids harbouring Arc 3'UTR downstream of a Renilla luciferase gene were generated and tested in a dual-luciferase reporter assay. To reveal the specific contribution of Arc 3'UTR splicing, the two introns contained in Arc UTR were omitted in a control construct.

Our results show that in neurons, upon BDNF induction, a luciferase construct harbouring Arc 3'UTR with introns is subjected to a significant upregulation in its expression levels compared to a non-intron control. Our analyses suggest that this BDNF- and Splicing-dependent induction occurs at the translational level, and that transcription of transacting factors is also required. Additionally, we find that eIF2 dephosphorylation, ERK, PKC and PKA activity are crucial to this process.

Overall, splicing of Arc 3'UTR exerts a dual and unique effect in fine-tuning Arc expression upon synaptic signalling: while inducing mRNA decay, to limit the time window of expression, splicing also elicits translation of the fast decaying mRNA. This apparent paradox likely serves to achieve a rapid yet limited burst of expression, a feature of Arc expression, which potentially underlies its role as a key sensor in synapse-specific homeostatic plasticity.

ENVIRONMENT-INDUCED NEUROPLASTICITY CHANGES IN KNOCK-IN MICE WITH THE HUMAN BDNF VAL66MET POLYMORPHISM

Ieraci Alessandro[1], Madaio Alessandro I[1], Mallei Alessandra[1], Lee Francis S[2], Popoli Maurizio[1]

[1]Laboratory of Neuropsychopharmacology and Functional Neurogenomics - Department of Pharmacological and Biomolecular Sciences University of Milano, Milano, Italy ~ Milano ~ Italy, [2]Department of Psychiatry, Weill Medical College of Cornell University, ~ New York ~ United States

Several clinical and preclinical studies have shown that physical exercise (PE) is an affordable and effective method to improve cognitive functions and emotional behaviors at all ages, even in the elderly. Positive effects of PE have been associated with enhanced brain plasticity, adult hippocampal neurogenesis and increased levels of brain-derived neurotrophic factor (BDNF). However, a substantial variability of individual responses to PE has been described. The reason for this is not known, but may be accounted, among others, by individual genetic variants. A common single nucleotide polymorphism (SNP) has been identified uniquely in the BDNF human gene (BDNF Val66Met) that leads to decreased BDNF secretion and has been associated with cognitive deficits and neuropsychiatric disorders. Recently, a knock-in mouse carrying the BDNF Val66Met polymorphism has been generated, which reproduces some of the principal phenotypes identified in Val66Met human carriers. In addition, BDNFMet/Met mice do not respond to antidepressant treatment with SSRIs. Despite the well-documented role of BDNF as mediator of the positive consequences of PE, it is presently unknown whether the BDNF Val66Met SNP moderates the degree to which an individual may benefit from PE.

To assess whether and how the BDNF Val66Met polymorphism influences the neurobiological effects stimulated by PE in the Val66Met knock-in adult male mice.

Wild-type (BDNFVal/Val) and homozygous BDNF Val66Met (BDNFMet/Met) male mice were housed in cage equipped with or without running wheels for 4 weeks. Behavioral modifications, hippocampal adult neurogenesis changes and gene expression variations were evaluated in PE and sedentary control mice.

We found that PE reduced the latency to feed in the novelty suppressed feeding and the immobility time in the forced swimming test in BDNFVal/Val but not BDNFMet/Met mice. PE-induced hippocampal neurogenesis was reduced in BDNFMet/Met mice compare to BDNFVal/Val mice. PE significantly increased total BDNF mRNA, and BDNF splice variants 1, 2, 4, 6 and mGluR2 in the dentate gyrus of BDNFVal/Val but not in BDNFMet/Met mice. BDNFMet/Met mice had lower basal BDNF protein levels in the hippocampus, which did not increase following PE treatment.

Overall these results indicate that, in adult male mice, the BDNF Val66Met polymorphism impairs the positive behavioral and neuroplasticity effects induced by PE.

ESSENTIAL ROLE OF ASTROGLIAL P75 NTR FOR LTP MAINTENANCE AND VISUAL RECOGNITION MEMORY

Vignoli Beatrice

EBRI-EUROPEAN BRAIN RESEARCH INSTITUTE ~ ROME ~ Italy

Brain-derived neurotrophic factor (BDNF) is the richest neurotrophin in the brain and is primarily produced by neurons, where the basal levels are promptly and intensely regulated by neuronal activity. Particularly, synaptic modifications induced by defined activity patterns, such as θ-burst-stimulation (TBS), are associated to an increase of BDNF production at both transcriptional and translational levels, which regulate spatio-temporal availability of the neurotrophin by means of an activity-dependent release mechanism.
Astrocytes express undetectable levels of BDNF and whether glial cells in response to patterned stimulation to modulate synaptic plasticity would recruit this neurotrophin for a functional re-use represents an attractive hypothesis. Our previous study indicates that this could be the case. We showed that p75NTR is expressed in astrocytes from perirhinal cortex and provides the molecular determinant for proBDNF uptake by these cells upon TBS-induced LTP; moreover, cortical astrocytes contain an endocytic compartment that is competent for proBDNF recycling. Taken together these findings suggest that astroglial p75NTR governs clearance and recycling of proBDNF, but the functional role of the neurotrophin brought by astrocytes remains unknown

Here we studied the physiological role of BDNF released by glial cells during synaptic potentiation and plasticity.

To address this issue, we generated tamoxifen-inducible conditional mutant mice selectively deleting astroglial p75NTR by crossing mice flanking exons 4-6 of the p75NTR gene with loxP sites with mice expressing the inducible version of the Cre recombinase CreERT2 under the control of the GLAST promoter. We recorded basal and stimulated (TBS: 100 Hz, four trains separated by 15 s) synaptic transmission in layer II/III of perirhinal cortex. We monitored LTP induction (60 min after TBS) and maintenance (180 min after TBS) in slices from control and floxed mice. Moreover we subjected floxed mice and control littermates to short- and long-term recognition memory in the object recognition task (ORT).

We demonstrated that deletion of p75NTR in astrocytes prevents proBDNF uptake and recycling in these cells precluding LTP maintenance. Moreover, ectopic expression of the neurotrophin in p75NTR -deficient astrocytes, which circumvent the failure of these cells to store and recycle proBDNF, restored the LTP deficit. Finally, mice deficient in astroglial p75NTR failed to recognize previously encountered objects in behavioral tests, an effect that is recovered by glial-specific expression of BDNF.

Overall, our data suggest that astrocytes act as a cellular source of BDNF, which is essential for LTP maintenance and higher-order activity of the perirhinal cortex, such as the visual recognition memory.

Symposium 9

TARGETING NEUROSTEROID PATHWAYS FOR THE TREATMENT OF TOURETTE SYNDROME AND IMPULSE CONTROL DISORDERS: FROM BENCH TO BEDSIDE.

Bortolato Marco

University of Kansas ~ Lawrence ~ United States

Ample evidence highlights the role of neuroactive steroids in the functional regulation of dopaminergic neurotransmission.

Capitalizing on these premises, our group has recently investigated the role of key enzymes in the neurosteroid synthesis pathway, such as 5-alpha reductase and 17-alpha hydroxylase, in Tourette syndrome (TS) and pathological gambling (PG), two neuropsychiatric disorders characterized by dysregulations of the dopaminergic system.

We studied the impact of 5-alpha reductase and 17-alpha hydroxylase inhibitors, in animal models capturing key phenotypic aspects of TS and PG. In particular, we focused on the prepulse inhibition of the acoustic startle and spontaneous tic-like responses in D1CT-7 mice (one of the best-validated models of TS) and probability discounting in rats (a model of decision making based on the same neuroeconomic tasks used in gambling activities). Furthermore, we tested the impact of some of these drugs on the severity of TS and PG in preliminary clinical studies.

Our preclinical findings indicate that the inhibitors of 5-alpha reductase and 17-alpha hydroxylase counter the effects of dopaminergic agonists in animal models of TS and PG. These results have been paralleled by preliminary clinical findings on both illnesses.

Our findings point to neuroactive steroids as novel potential therapeutic targets for impulse-control disorders.

NEONATAL NEUROACTIVE STEROID LEVELS ALTERATION: LONG TERM-EFFECTS ON BEHAVIOUR IN RATS.

Darbra Sonia[3], Modol Laura[3], Llidó Anna[3], Vallée Monique[2], Casas Caty[1], Pallarès Marc[3]

[1]Institut de Neurociències and Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona ~ Bellaterra (Cerdanyola del Vallès) ~ Spain, [2]INSERM Unit 862 ~ Bordeaux ~ France, [3]Departament de Psicobiologia i Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona ~ Bellaterra (Cerdanyola del Vallès) ~ Spain

The maintenance of endogenous neuro active steroid (NS) levels has been postulated to be of importance for the maturation of the CNS and particularly for the hippocampus.

We have focused our work on the long-term effects of neonatal NS levels alteration on the adult response to novel environmental stimuli, anxiety-related behaviors, learning and processing of sensory inputs (prepulse inhibition).

Neonatal NS levels were altered by allopregnanolone and finasteride administration, from PD5 to PD9. We have also pay due care and attention the nature of the control group used to investigate the possible effects of postnatal manipulations.

Our data show that neonatal NS levels alteration increases emotional reactivity in situations of stress or conflict in the adolescent age, deteriorates novel object recognition and deteriorates PPI and produce a behavioral profile consistent with anxiety reduction in the elevated plus maze in adulthood, supporting the relevance of postnatal NS levels for brain development and for adult behaviour.
However, the mechanisms by which these developmental changes take place are still unknown. One possible mechanism could be through the main allopregnanolone modulation ionotropic target, the GABAAR. Both synaptic and extrasynaptic GABAA receptors represent targets for the actions of NS (Belelli et al., 2009). We conducted different experiments in order to study the mediation of GABAA receptor on the long-term effects of neonatal NS levels alteration on behavior. First, we have showed that allopregnanolone effects on novelty-induced activity, anxiety-like behavior and prepulse inhibition were abolished by coadminstration PregS, a GABAA negative modulator used as a pharmacological tool. Moreover, finasteride administration modifies neonatal and adult expression of the α4 and δ GABAAR subunits, which is accompanied by an altered behavioural response to progesterone administration, even in adulthood. Finally, alterations in the neonatal NS levels by exogenous allopregnanolone administration or by finasteride administration change the developmental hippocampal expression and protein abundance profile of the the neuron-specific K+/Cl− co-transporter 2 (KCC2, a chloride exporter) during neonatal period in vivo.

Together these results support the relevance of postnatal NS levels for brain development and for adult affective behaviour and suggest that neonatal NS alteration effects on behavior could be mediated in part through a GABAA receptor modulation.

EFFECT OF FINASTERIDE TREATMENT IN THE NERVOUS SYSTEM: CLINICAL AND EXPERIMENTAL OBSERVATIONS.

Melcangi Roberto Cosimo

Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano ~ Milano ~ Italy

Finasteride is a commercially available 5alpha-reductase (5alpha-R) reversible inhibitor. This molecule blocks the conversion of progesterone and testosterone into dihydroprogesterone and dihydrotestosterone, respectively.
These neuroactive steroids, as weel as further their metabolites, are important mediators for many physiological processes in the nervous system (NS), affecting mood, behavior, reproduction, and cognition. Moreover, neuroactive steroids act as protective agents in different experimental models of neurodegeneration. Therefore, the enzymatic conversion mediated by 5alpha-R exerts a crucial role in NS.
Despite that, the effects exerted in the NS by the chronic treatment with finasteride, and particularly by its withdrawal have been poorly considered. This aspect could be important, particularly because observations performed in a subset of man taking finasteride for male pattern hair loss show sexual dysfunction as well as anxious/depressive symptomatology. Very important, persistent sexual side effects as well as depression persist despite the discontinuation of the treatment (i.e., PFS patients).

A possible hypothesis to explain nervous symptoms after finasteride treatment might be an impairment of the levels of neuroactive steroids.
To this aim, neuroactive steroids levels were evaluated in plasma and cerebrospinal fluid (CSF) of male PFS patients. Moreover, the effects of chronic treatment with finasteride and its withdrawal on neuroactive steroids levels and expression of their receptors were evaluated in male rats. Neuroactive steroid levels were assessed in patients and animal model by liquid chromatography tandem mass spectrometry. Male rats were treated daily for 20 days with 3mg/kg of finasteride and at the end of chronic treatment and after a month of withdrawal neuroactive steroid levels were assessed in plasma, CSF, cerebellum, cerebral cortex and hippocampus. Moreover, the expression of androgen, estrogen and progesterone receptor as well as of GABA-A receptor subunits (i.e., alpha2, alpha4, beta3, gamma2 and delta subunits) were analyzed.

Data obtained in PFS patients show altered levels of neuroactive steroids both in CSF and plasma. Data obtained in the animal model indicate that, after chronic treatment, depending on the compartment considered, alteration in the levels of neuroactive steroids as well as in expression of steroid receptor, such as androgen receptor, and in beta3 subunit of GABA-A receptor were observed. At the withdrawal some of these effects persisted and different changes in neuroactive steroid levels, and in the expression of receptors were detected.

Altogether these findings suggest that the block of the enzyme 5alpha-reductase by finasteride treatment may have broad consequences for the brain.

NEUROSTEROIDS DURING DEVELOPMENT: IMPLICATIONS FOR GABA-A RECEPTOR FUNCTION AND STRESS SENSITIVITY IN ADULTHOOD

Porcu Patrizia[1], Locci Andrea[2], Talani Giuseppe[1], Licheri Valentina[2], Lallai Valeria[2], Dazzi Laura[2], Sanna Enrico[2], Concas Alessandra[2]

[1]Neuroscience Institute, National Research Council of Italy (CNR) ~ Cagliari ~ Italy, [2]Dept. of Life and Environment Sciences, Section of Neuroscience and Anthropology, University of Cagliari ~ Cagliari ~ Italy

Exposure of developing female rats to estradiol during the perinatal period affects brain sexual differentiation and induces a long-lasting dysregulation of the gonadal axis with altered brain concentrations of progesterone and its neuroactive metabolite allopregnanolone.

Allopregnanolone is a potent modulator of GABA-A receptor expression and function; we thus evaluated whether the marked and persistent decrease in its concentrations, induced by neonatal estradiol treatment, alters GABA-A receptor expression and function, stress sensitivity, and behavior in adult female rats.

On the day of birth, pups received a single s.c. administration of beta-estradiol 3-benzoate (EB; 10micrograms in 50microliters of sesame oil) or vehicle (controls). Experiments were performed in 60-120 days old rats.

Neonatal EB administration markedly decreased allopregnanolone levels in the cerebral cortex (-86%), hypothalamus (-55%) and hippocampus (-47%) of adult rats. This effect was associated with increased expression of extrasynaptic alpha4-beta-delta GABA-A receptors and decreased expression of synaptic alpha1/alpha4-beta-gamma2 GABA-A receptors in the hippocampus. As a result, modulation of GABAergic tonic currents recorded in DG granule cells by THIP was increased in EB-treated rats vs. controls, with a larger holding current shift and a 26% enhancement of noise variance. EB treatment altered GABAergic phasic currents with a decrease in decay time and an increase (+76%) in frequency. Neonatal EB treatment increased dominant behaviors (duration: +142%, frequency: +121%) in the resident-intruder test, while locomotor activity, anxiety- and mood-related behaviors, as well as seizures sensitivity were not affected. Moreover, neonatal EB treatment induced a greater enhancement in the neurosteroid response to stress (5-fold), as well as in the extracellular dopamine concentrations (4-fold) in the prefrontal cortex of adult EB-treated rats exposed to foot-shock stress; this latest effect was normalized by restoring allopregnanolone concentrations.

These results suggest that neonatal estradiol exposure plays a major role in regulating brain allopregnanolone concentrations, expression and function of synaptic and extrasynaptic GABA-A receptors, agonistic behavior and stress sensitivity during adulthood. Given that allopregnanolone has been involved in stress homeostasis, the persistent decrease in its concentrations, induced by estradiol, may account for the heightened stress response. Likewise, the increased expression of hippocampal alpha4-beta-delta GABA-A receptors may represent a homeostatic response to counteract the persistent decrease in allopregnanolone levels induced by estradiol. Thus, neonatal estradiol treatment alters inhibitory synaptic circuits and behavior in adult rats, in addition to its effects on brain sexual differentiation.

Supported by Banco di Sardegna Foundation (2012.0255).

Symposium 10

REGULATION OF SYNAPTIC CONNECTIVITY BETWEEN GABAERGIC INTERNEURONS AND NG2 CELLS DURING CORTICAL OLIGODENDROGENESIS

Angulo Maria Cecilia

INSERM U1128 ~ Paris ~ France

Oligodendrocyte precursor cells, also named NG2 cells, constitute the major source of myelinating oligodendrocytes in the brain. In the developing neocortex, NG2 cells transiently receive a major synaptic input from GABAergic interneurons (Vélez-Fort et al. 2010; Balia et al., 2015). However, the operating modes and functions of these neuron-glia synapses are unknown.

We recently examined the properties of individual interneuron-NG2 cell synapses from the subcellular to the network level during the critical period for oligodendrogenesis in the somatosensory cortex.

We used paired recordings, holographic photolysis for circuit mapping and immunohistochemistry.

We unexpectedly found that the GABAergic innervation of OPCs by cortical interneurons form a structured synaptic network that is temporally and spatially regulated in coordination with the onset of oligodendrogenesis. Fast-spiking interneurons are highly connected to NG2 cells whereas Non-Fast-spiking interneurons are poorly connected to these cells. These two different types of interneurons discriminate NG2 cell postsynaptic sites, targeting anatomically segregated subcellular domains, containing distinct GABAA receptors. NG2 cells thus compartmentalize input regions according to the identity of the interneuron. This subcellular arrangement of presynaptic inputs is extended at the network level. Holographic photolysis revealed that interneuron-NG2 cell connections exhibit more local connectivity maps than interneuron-pyramidal cell connections, forming a specific network characterized by a local microarchitecture. Interneuron-NG2 cell synaptic connectivity is transient and reaches a peak at post-natal day 10, coinciding with a switch to a massive NG2 cell differentiation onto oligodendrocytes (Orduz et al., 2015).

In conclusion, NG2 cell synaptic connectivity is highly regulated in time and space during cortical development. This regulation is correlated with important oligodendrocyte developmental processes, suggesting the implication of these neuron-glia synapses in the fate of NG2 cells.

MOLECULAR MECHANISMS IN OLIGODENDROGLIOMA INFILTRATION AND MALIGNANCY

Appolloni Irene[1], Barilari Manuela[1], Malatesta Paolo[2]

[1]IRCCS AOU San Martino IST ~ Genova ~ Italy, [2]Università di Genova ~ Genova ~ Italy

High grade oligodendrogliomas are extremely invasive tumors of the central nervous system composed of oligodendrocyte-like cells. Despite all the efforts made to find an effective therapy, they are still incurable mainly because current treatments fail to fully eradicate the cells infiltrated in the brain parenchima that, acting as stem cells, can proliferate inducing relapses. A better understanding of the molecular and cellular mechanisms at the basis of the migratory ability of these cells is a crucial step to design more effective therapies.

We aim to identify the molecular mechanism underlying glioma invasivity. Defining the molecules that are necessary to a glioma cells for migrating inside the healthy parenchyma, ignoring cell-cell contact inhibition.

We exploited an in vivo murine model of oligodendroglioma, we previously developed. Tumors were induced by transducing PDGF-B in embryonic brain, therefore mimicking a deregulation of PDGF signalling often seen in human oligodendrogliomas. Modulation of genes originally identified by a microarray expression analysis comparing migrating and non-migrating glioma cells was used to assess their actual role in migration both in vitro and in vivo.

We demonstrated that PDGF-B overexpression is required to confer in vivo infiltrating potential to tumor cells by overcoming the mechanism of cell-cell contact inhibition of migration. Moreover, we noticed that a re-localization of cadherins, reminiscent of the E- to N-cadherin switch seen during the epithelial to mesenchymal transition, takes place in these malignant cells. We demonstrated that R-cadherin overexpression is induced by PDGF-B, and is necessary to destabilize the N-cadherin dependent adherent junctions (AJs), releasing cells from cell-cell contact inhibition. Strikingly, R-cadherin silencing restores both cell-cell contact inhibition and localization of N-cadherin in AJs thus preventing tumor cells from migrating through the brain parenchyma and impairing their tumorigenic ability. Moreover, a survival analysis on publically available data shows that the expression level of R-cadherin in glioblastoma patients is correlated to shorter life expectancy.

Altogether, these results suggest that R-cadherin is necessary for oligodendroglioma cells migration and could therefore represent a valuable target for oligodendroglioma therapies.

BALANCING SELF-RENEWAL AND DIFFERENTIATION OF THE OLIGODENDROCYTE PROGENITOR POOL: INSIGHTS INTO CELL INTRINSIC REGULATORY MECHANISMS

Boda Enrica

Dept. of Neuroscience Rita Levi Montalcini, Neuroscience Institute Cavalieri Ottolenghi, University of Turin ~ Turin ~ Italy

Oligodendrocyte progenitor cells (OPCs) persist in the adult Central Nervous System (CNS) and guarantee oligodendrocyte turnover and myelin repair throughout life.

It remains obscure how OPCs avoid exhaustion during adulthood and whether, similar to neural stem cells, they include distinct populations/stages intrinsically committed to self-maintain or to produce a differentiating progeny.

To address these issues, by in vivo fate-mapping approaches we examined the phenotype of OPCs during mitosis and the fate of their daughter cells at distinct time points after cell birth.

We found that distinct transcription factors are expressed in segregated subsets of dividing OPCs in the mouse adult and juvenile cerebral cortex. Such subsets produce cell progenies with different fates. Further, we showed that a fraction of dividing OPCs gives rise to asymmetric daughter cell pairs including sister OPCs with diverse early immunophenotypic profiles and fates (i.e. acquisition of premyelinating markers or maintenance of progenitor features, including the expression of NG2, PDGFRa and the ability to undergo re-proliferation). Sister OPC heterogeneity appears as early as cells exit cytokinesis, suggesting that, similar to stem cells, a subset of the dividing OPCs can undergo asymmetric division. Interestingly, although molecules such as NG2 and PDGFRa expressed in the mother cells do not segregate asymmetrically during OPC mitosis, OPCs express a repertoire of classical molecular regulators of the cell division modality operating in neural stem cells, including cell fate determinants and polarity machineries. The generation of asymmetric OPC pairs appears significantly reduced in the aged brain.

Our data point to the existence of cell intrinsic mechanisms that finely tune the OPC turn-over to preserve the progenitor pool while assuring the production of new oligodendrocytes in the intact adult brain. Alteration of these mechanisms may contribute to the hamper oligodendrogenesis and myelin repair in the aged CNS.

NEW MECHANISMS REGULATING OLIGODENDROGLIAL DIFFERENTIATION: FOCUS ON THE GPR17 RECEPTOR AND RELATED MICRORNAS

Lecca Davide[1], Marangon Davide[1], Coppolino Giusy Tindara[1], Meraviglia Veronica[2], Bonfanti Elisabetta[1], Fumagalli Marta[1], Rosa Patrizia[2], Furlan Roberto[3], Abbracchio Maria Pia[1]

[1]Laboratorio di Farmacologia Molecolare e Cellulare della Trasmissione Purinergica, Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Università degli Studi di Milano ~ Milano ~ Italy, [2]Istituto di Neuroscienze, CNR ~ Milano ~ Italy, [3]Unità di Neuroimmunologia, Istituto di Neurologia Sperimentale (INSpe), Divisione di Neuroscienze, Ospedale San Raffaele ~ Milano ~ Italy

Oligodendrocyte precursor cells (OPCs) are the primary source of myelinating oligodendrocytes in both the developing and adult central nervous system (CNS). During early differentiation stages, they start to express GPR17, a G protein-coupled receptor that through its signalling contributes to oligodendrocyte differentiation. However, after this stage, GPR17 has to be down-regulated to allow cells' terminal maturation. Our in vitro and in vivo data indicate that alterations or pathological dysregulation of this strict time-regulated expression pattern can lead to myelination impairment.

Our project was aimed at identifying the intrinsic and extrinsic signals switching on/off the expression of GPR17 during the physiological differentiation of oligodendrocytes, and understanding how these mechanisms can influence myelination under pathological conditions.

To address these issues, we analysed the effect of different stimuli on the activity of GPR17 promoter by means of a luciferase reporter assay in Oli-neu cells, an immortalized oligodendroglial cell line, transfected with a putative promoter sequence of GPR17. Then, to assess if also post-transcriptional regulation could affect GPR17 expression, we performed an in silico analysis in databases of microRNAs (miRNAs) and we identified potential targets. The effect of a candidate miRNA was evaluated on rat primary OPCs (see also presentation by Marangon et al, this meeting).

Our reporter assay showed that exposure of Oli-neu cells to a medium conditioned by cortical neurons led to significant induction of GPR17 promoter activity, suggesting the release of a neuronal activator molecule. Indeed, axon-glia communication and extracellular signals can profoundly influence the expression pattern of oligodendrocytes, and this is responsible of their behavior, both in physiological and pathological conditions.
Moreover, we have recently identified miR-X, a miRNA that putatively targets both GPR17 and other key players of oligodendroglial differentiation. Interestingly, both its forced expression and its silencing strongly altered OPC maturation in culture as demonstrated by significant changes in the number of cells expressing the myelin basic protein MBP. Furthermore, the levels of miR-X were altered in both spinal cord from mice subjected to experimental autoimmune encephalomyelitis (EAE) and in cerebrospinal fluid of MS patients, suggesting a possible role as a hallmark of the disease.

Globally, these data suggest that GPR17 expression in oligodendrocytes is regulated by both genic and epigenetic mechanisms and dysregulation of these mechanisms contributes to defective remyelination. Understanding the molecular link between miR-X, GPR17 and other myelin genes will provide novel therapeutic means to enhance endogenous CNS reparative capabilities in neurodegenerative diseases.

Symposium 11

THE SEROTONIN SYSTEM IN L-DOPA-INDUCED DYSKINESIA, FROM RATS TO HUMANS

Björklund Anders

Wallenberg Neuroscience Center, Division of Neurobiology, Lund University, 221 84 Lund, Sweden

An increasing body of experimental evidence suggests that the serotoninergic neurons play a major role in the production of L-DOPA-derived dopamine when most of dopaminergic neurons have degenerated, and that un-regulated release of dopamine from serotoninergic neurons is responsible for the appearance of L-DOPA-induced dyskinesia (LID) in animal models of Parkinson's disease (PD). In fact, pre-clinical studies show that silencing of 5-HT1 receptors by selective ligands can suppress LID both in 6-OHDA-lesioned rat and MPTP-treated monkey models of PD.

The pre-clinical evidence led to a number of recent clinical investigations, which provided support to the idea that the serotonergic system is involved in the appearance of LID also in PD patients.

In this talk we will provide an overview of both animal and human studies, and we will discuss the serotonin system as a possible target for anti-dyskinetic therapies.

ALTERATION OF NMDA RECEPTOR SUBUNITS AS SYNAPTIC TRAIT OF L-DOPA-INDUCED DYSKINESIA: MOLECULAR MECHANISMS AND EFFECT OF ELTOPRAZINE TREATMENT

Gardoni Fabrizio

University of Milano ~ Milano ~ Italy

Levodopa-induced dyskinesias (LIDs) are major complications in the pharmacological management of Parkinson's disease (PD). Abnormal glutamatergic transmission in the striatum is considered a key factor in the development of LIDs.

Characterization of NMDA receptor GluN2A/GluN2B subunit ratio as a common synaptic trait in rat and primate models of LIDs and in dyskinetic PD patients.

We used a biochemical fractionation approach to analyse the molecular composition of the excitatory synapse in animal models of PD and LIDs.

The modulation of synaptic NMDA receptor composition by a cell-permeable peptide interfering with GluN2A subunit interaction with the scaffolding protein PSD-95 leads to a reduction in the dyskinetic motor behavior in the two animal models of LIDs. Here we show that chronic treatment with the 5-HT1A/1B agonist eltoprazine is able to restore the molecular composition of the excitatory synapse in L-DOPA treated dyskinetic ras. In particular, the analysis of NMDA receptor subunit content in the postsynaptic fraction shows a normalization of GluN2A/2B subunits levels in the L-DOPA plus eltoprazine treated dyskinetic rats. Moreover, in the striatal postsynaptic fraction we also demonstrated that eltoprazine normalizes the state of D1R/DARPP-32 signaling as measured by striatal pGluR1845 levels in treated animals.

Overall, the normalization of both D1 signaling pathway and the glutamatergic NMDA receptors can explain the anti-dyskinetic effect of eltoprazine treatment.

MODULATION OF SEROTONERGIC TRANSMISSION IN L-DOPA-INDUCED DYSKINESIA: BEHAVIORAL, MOLECULAR, AND SYNAPTIC MECHANISMS

Picconi Barbara

Fondazione Santa Lucia, IRCCS ~ Roma ~ Italy

Long-term treatment of Parkinson's disease (PD) patients with L-3,4-dihydroxyphenylalanine (L-DOPA) is, at present, the most effective therapy for motor symptoms relief. Major side effects of chronic administration of L-DOPA are abnormal involuntary movements (AIMs) known as L-DOPA induced dyskinesias (LIDs). Many studies support the involvement of raphe-striatal serotonin neurons as crucial for the onset of LIDs, as they are able to convert exogenous L-DOPA to dopamine (DA) but lack the cellular components for the feedback machinery to regulate synaptic DA levels and, therefore, may contribute to the pulsatile stimulation of DA receptors in PD treatment.

Despite this evidence little is known about the molecular and electrophysiological consequences on this serotonin receptor modulation at striatal neurons. The aim of this study is to clarify the possible restorative effects on the bidirectional striatal synaptic plasticity underlying the anti-dyskinetic effect of the 5-HT1A/1B agonist.

Here we focus on the behavioural (motor and non motor effects) and electrophysiological effects of a mixed 5-HT1A/1B receptor agonist, eltoprazine, in unilaterally 6-OHDA-lesioned rats modelling PD and in parkinsonian rats developing LIDs.

The present study demonstrates that activation of 5-HT1A/1B receptors reduces LIDs via the restoration of LTP and depotentiation in a sub-set of striatal MSNs.This recovery is associated with the normalization of D1 receptor-dependent cAMP/PKA and ERK/mTORC signaling pathways, and the recovery of NMDA receptor subunits balance, indicating these events as key elements in LIDs induction. Moreover, we have analyzed whether the alteration of the serotonergic system might affect motor and cognitive behaviors.

The monomeric GTP-binding protein Rhes influences the number of midbrain Dopaminergic neuron and the motor disturbances associated to L-DOPA in 6OHDA lesioned mice

Alessandro Usiello and Francesco Napolitano

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples (SUN), Caserta, Italy. CEINGE Biotecnologie Avanzate

Rhes is a small GTP-binding protein highly enriched in the striatum, developmentally regulated by thyroid hormone, and by dopamine (DA) innervation in adult rat brain. Within the striatum, *Rhes* mRNA is localized in DA D1R- and D2R-bearing medium-sized projection neurons, as well as in large aspiny cholinergic interneurons, where it modulates DA-dependent transmission and signaling. Previous findings that Rhes, a striatal-enriched small G protein, accounts for the unique neuropathology of Huntington's disease by enhancing mutant huntingtin sumoylation and toxicity. Interestingly, Rhes is also able to bind and activate mTOR.

We first evaluated *Rhes* mRNA expression pattern in midbrain regions, then we investigated the potential involvement of this GTPase in regulating the number and density of DA neurons and nigrostriatal-sensitive behavior during aging. Finally, we assessed whether Rhes might influence the striatal mTOR-dependent L-DOPA-induced dyskinesia in the hemi-parkinsonian *Rhes* KO mouse model.

Radioactive *in situ* hybridization was assessed in adult mice. Beam-walking was executed in 3-,6- and 12-month-old mice. Immunohistochemical staining and stereological analyses of midbrain tyrosine hydroxylase (TH)-positive neurons were performed in 6- and 12-month-old mice. Unilaterally 6-OHDA-lesioned mice were analysed for the anti-akinetic effect of L-DOPA before and 1 h after the first injection of 10 mg/kg L-DOPA to the cylinder test. 6-OHDA-lesioned mice were treated for 9 consecutive days with one injection per day of 10 mg/kg L-DOPA plus benserazide (20 mg/kg). AIMs were assessed at day 3, 6 and 9 by an observer blind to the mouse treatment.

Rhes mRNA is expressed in TH-positive neurons of substantia nigra and ventral tegmental area. Moreover, lack of Rhes leads to roughly 20% of nigral TH-positive neuronal loss in both 6- and 12-month-old mutants, when compared to their age-matched controls. *Rhes* mutants display subtle alterations in motor coordination, as measured by beam-walking test. Moreover, 6-OHDA-lesioned *Rhes* KO mice show a significant reduction of L-DOPA-induced dyskinesia, via enhancement of the striatal mTOR signaling, without affecting the therapeutic improvement of forelimb movement.

Our findings indicate a subtle although significant role of Rhes in regulating the number of TH-positive neurons of substantia nigra and nigrostriatal–sensitive behavior. In addition, given the negligible levels in peripheral tissues, drugs blocking Rhe-mTOR interactions may have much less potential for adverse effects, consistent with the lack of major abnormalities in mutant mice.

Symposium 12

MOTOR NEURON-IMMUNE SYSTEM CROSS-TALK IN ALS: EVIDENCE AND CONSEQUENCES ON THE DISEASE

Bendotti Caterina, Trolese Maria Chiara, Bosani Beatrice, Verderio Mattia, Nardo Giovanni

IRCCS-Mario Negri Institute ~ Milano ~ Italy

Amyotrophic Lateral Sclerosis is characterized by a progressive, and more or less rapid loss of motor functions and premature death due to respiratory failure. The derangment of the motor neurons (MNs) is the cause of this devastating disease, which in 10% of the cases is inherited but indistinguishable clinically and neuropathologically from sporadic forms. The MN in ALS is largely exposed to toxic factors both intracellular like protein aggregates, and extracellular like proinflammatory environment, thus making its defense challenging and complex. Recently we demonstrated in two mouse models of ALS with different disease severity, that the more the MN is able to up-regulate molecules of the immune response the more slowly the disease progresses. In particular, we found a remarkable upregulation of the molecules of MHCI pathway in the spinal MNs of slow progressing trangenic SOD1G93A mice at the disease onset.

The aim of this study was to examine in detail the expression of the MHCI pathway at the central and peripheral nervous system of SOD1G93A mice during the disease course and how this correlate with the antigen presentation to CD8+ T cells both at the central and peripheral level.

We use the immunohistochemical analysis and real time PCR in the spinal cord, peripheral nerves and muscles of SOD1G93A mice at different stages of the disease to examine the expression level and distribution of the MHCI pathway components and the T lymphocytes.

MNs and surrounding glial cells (microglia, olygodendrocytes but not astrocytes) show the activation of the immunoproteasome subunit (LMP7), MHCI and Beta2microglobulin (B2m) at very early stages of the disease. Notably, while the immunostaining of LMP7 and Beta2m were highly increased in the perikaria of MNs and motor axons, the MHCI immunoreactivity was increased exclusively in the motor axons and neuromuscular junction of SOD1G93A mice during the disease course. Consistently, we found CD8+ T lymphocytes infiltrates in the spinal cord, sciatic nerve and muscle of SOD1G93A suggesting the interaction of MNs with cytotoxic T cells through MHCI.

These data point out that the activation of the adaptive immune system molecules both at central and peripheral level may take part in the pathogenesis and/or progression of ALS. Studies are ongoing to investigate the beneficial or detrimental effect of this immune response on the disease course of SOD1G93A mice cross-bred with mice deficient of the MHCI pathway.

This work is supported by the Thierry Latran Foundation and AriSLA and Euro-MOTOR.

GROUP I METABOTROPIC GLUTAMATE RECEPTORS AND NEUROTOXICITY IN AMYOTROPHIC LATERAL SCLEROSIS

Milanese Marco[1], Bonifacino Tiziana[1], Cattaneo Luca[1], Musante Ilaria[2], Gallia Elena[1], Melone Marcello[3], Conti Fiorenzo[3], Puliti Aldamaria[2], Bonanno Giambattista[4]

[1]Dept. of Pharmacy, Pharmacology and Toxicology Unit, University of Genova ~ Genoa ~ Italy, [2]Dept. of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, Medical Genetics Unit, University of Genova and Gaslini Institute ~ Genoa ~ Italy, [3]Dept. of Experimental and Clinical Medicine, Neuroscience and Cell Biology Unit, Università Politecnica delle Marche and Center for Neurobiology of Aging, INRCA IRCCS ~ Ancona ~ Italy, [4]Dept. of Pharmacy, Pharmacology and Toxicology Unit, and Center of Excellence for Biomedical Research, University of Genova ~ Genoa ~ Italy

Amyotrophic lateral sclerosis (ALS) is an adult-onset disease characterized by degeneration of motoneurons (MNs) resulting in muscle weakness, paralysis and death for respiratory failure. The etiology of ALS remains unknown and the mechanism of MN damage and death has been ascribed to several causes. Glutamate (Glu)-mediated excitotoxicity is still one major factor accountable for MNs neurodegeneration. We published data showing the presence of excessive Glu exocytosis in the spinal cord of the SOD1G93A mouse model of ALS. This abnormal Glu release was induced by different mechanisms, including the activation Group I metabotropic Glu receptors (mGluR1, mGluR5). mGluR1 and mGluR5 represent the only excitatory mGluRs and are actively involved in the regulation of important cellular processes altered in ALS moreover, these receptors have been found to be largely over-expressed in different experimental model of ALS.

The major goal of our study was to investigate the effect of the mGluR1 or mGluR5 down-regulation in ALS.

We generated three different SOD1G93A mouse strains: SOD1G93AmGluR1+/-, SOD1G93AmGluR5+/- and SOD1G93AmGluR5-/-, carrying half expression of mGluR1, half expression of mGluR5 and total absence of mGluR5, respectively. Life span, motor abilities, MNs preservation, mitochondrial damage, oxidative stress markers, astrogliosis and microglia activation, receptor expression and glutamate release were investigated to characterize double mutant mice compared to the SOD1G93A ALS model.

Genetic knock-down of mGluR1 in SOD1G93A mice showed a positive impact on disease progression and life span. Behavioral improvements were paralleled by MN preservation, reduced astrogliosis and microglia activation, normalization of oxidative stress markers, reduced mitochondrial damage and decrease of the excessive Glu-induced Glu release. Half expression of mGluR5 in SOD1G93A mice showed delayed pathology onset and a prolonged life span. Surprisingly, these results were not accompanied by improved motor performances registered in behavioral tests. However we found a significant preservation of spinal motor neuron in the late phase of the disease and a normalized Glu-induce Glu release triggered by the activation of Group I metabotropic Glu receptors. When studying the SOD1G93A mice knockout for mGluR5 we got even more striking results in terms of prolonged survival probability. The life span amelioration was also accompanied by motor skills amelioration.

Our findings demonstrate that both mGluR1 and mGluR5 down-regulation has a significant impact in-vivo on ALS clinical outcome and provide a rationale for pharmacological approaches based on the selective block of Group I mGluRs.

THE INVOLVEMENT OF MUSCLE IN SBMA AND ALS

Pennuto Maria

Dulbecco Telethon Institute ~ Trento ~ Italy

Motor neuron diseases, such as spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS), are characterized by the progressive loss of motor neurons and skeletal muscle weakness, fasciculation, and atrophy. SBMA is caused by expansion of a polyglutamine tract in the androgen receptor (AR). ALS can be both sporadic and familial. Skeletal muscle is emerging as a critical component of SBMA and ALS pathogenesis. However, the impact of muscle dysfunction in these disorders remains to be clarified.

Here, we tested the hypothesis that dysregulation of AR signaling plays a critical role in SBMA and ALS pathogenesis. We analyzed muscle pathology in SBMA knock in mice. Moreover, we investigated the effect of specific diet regime in SBMA. Finally, we investigated the effect of alteration of AR signaling in ALS pathogenesis.

We performed histopathological analysis of skeletal muscle in SBMA knock in and ALS (SOD1-G93A) mice, surival analysis, behavior analysis, biochemistry analysis.

We have obtained evidence that polyglutamine-expanded AR directly affects skeletal muscle to cause SBMA. In SBMA, we observed a shift from glycolytic to oxidative metabolism in muscle, which was associated with atrophy that occurred in the absence of overt spinal cord pathology. We provided the mice with a special diet, and we observed an amelioration of muscle pathology and phenotype. Moreover, we obtained evidence that alteration of AR signaling in muscle may play a role also in ALS. In ALS, we found that stimulation of endogenous AR with synthetic androgens results in aggregation of AR and enhancement of muscle pathology in castrated mice. Importantly, normal AR accumulated in forms of aggregates in the muscle of mutant SOD1 mice upon androgen stimulation, raising the intriguing possibility that there is a genetic interaction between AR and mutant SOD1. These data support the idea that dysregulation of AR signaling is a key component in SBMA and ALS.

Skeletal muscle is a key component of motor neuron diseases, such as SBMA, and it plays an important role also in ALS. As skeletal muscle is an easier therapeutic target compared to spinal cord, novel therapeutic strategies may be designed to target muscle cells rather than motor neurons.

THE PROTEIN QUALITY CONTROL SYSTEM IN MOTONEURON DISEASES

Rusmini Paola[1], Crippa Valeria[2], Cicardi Maria Elena[1], Polanco Maria Josefa[3], Meroni Marco[1], Cristofani Riccardo[1], Pennuto Maria[3], Galbiati Maria Rita[1], Poletti Angelo[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, and Centre of Excellence on Neurodegenerative Diseases, Università degli studi di Milano ~ Milano ~ Italy, [2]Laboratorio di Neurobiologia Sperimentale, Istituto Neurologico Nazionale C. Mondino, Pavia ~ Pavia ~ Italy, [3]Dulbecco Telethon Institute Lab of Neurodegenerative Diseases, Centre for Integrative Biology (CIBIO), Università degli Studi di Trento, Trento, Italy ~ Trento ~ Italy

Spinal and bulbar muscular atrophy (SBMA) is a motoneuronal diseases caused by an elogated polyglutamine (polyQ) tract in the androgen receptor (AR). The polyQ expansion causes the AR protein to misfold and the binding with the ligand testosterone triggers a cascade of events, including ARpolyQ aggregation that led to motoneuron death. The intracellular accumulation of misfolded ARpolyQ both altered the protein quality control system (PQC) and impaired the protective mechanisms deputed to refolding and clearance of misfolded proteins. In PQC, the molecular chaperones allow the refolding or the clearance of the misfolded proteins through the Ubiquitin Proteasome system (UPS) or the autophagic pathway. Moreover, emerging evidence reveal that ARpolyQ toxicity is not related only to motoneuron degeneration but also skeletal muscle damage plays a primary role in SBMA.

The aim of the study was both to unravell the contribution of PQC in SBMA and to find molecular and pharmacological approaches for modulating PQC as potential therapeutic target.

Western blot and filter retardation assay were used to analyse the biochemical properties of ARpolyQ and the protein level of PQC markers. RT-qPCR was used to quantify the mRNA expression of PQC genes in presence of ARpolyQ.

In SBMA motoneuronal cell line, we demonstrated that both UPS and autophagic pathway are impaired or blocked, leading to ARpolyQ accumulation into the aggregates. Moreover, analysis in SBMA animal model showed that in the spinal cord and in the skeletal muscle, the PQC could differ considerably in how degrading the mutant and misfolded ARpolyQ.
In these conditions of PQC impairment we tested, in SBMA cell model, the overexpression of the small heat shock protein B8 (HspB8), involved in the autophagic pathway. HpB8 led to the autophagic removal of misfolded ARpolyQ, restorating the intracellular autophagic flux. Interestingly, we found that trehalose, a known autophagic stimulator, was able to induce the HspB8 expression and to facilitate the ARpolyQ clearance. Then, we tested the combined treatment of trehalose with Bicalutamide, an antiandrogen. Bicalutamide is able to slow down AR nuclear translocation and to retain it into the cytoplasm, where the autophagic pathway is active. Bicalutamide and trehalose showed synergic activity in the degradation of ARpolyQ.

The PQC plays a crucial role in SBMA, the modulation of its activity with trehalose and Bicalutamide might be a promising approach for this no cure disease.

GRANTS: Fondazione AriSLA; Fondazione Cariplo; AFM Telethon France; Regione Lombardia; UNIMI; Telethon Italy.

Symposium 13

KIDINS220/ARMS IS A FUNCTIONAL MEDIATOR OF NEUROTROPHIN SIGNALING PATHWAYS IN NERVOUS SYSTEM DEVELOPMENT, MATURATION AND PLASTICITY

Cesca Fabrizia

Istituto Italiano di Tecnologia ~ Genova ~ Italy

Kidins220 (Kinase D interacting substrate of 220 kDa)/ ARMS (Ankyrin Repeat-rich Membrane Spanning) is a membrane protein highly expressed in the nervous system. It acts as a signalling platform, establishing functional interactions with several membrane receptors and ion channels, including Trk- and p75 neurotrophin receptors, AMPA and NMDA glutamate receptors, and neuronal voltage-gated (Nav) sodium channels. Because of its pleiotropic interactions, Kidins220 modulates several aspects of neuronal physiology including survival and differentiation, synaptic plasticity and network activity. Not surprisingly, full Kidins220 knockout (Kidins220-/-) mice do not survive birth due to severe neural and cardiac abnormalities. Dysregulation of Kidins220 levels has been linked to the onset of Alzheimer's disease and of cancers of nervous system origin such as glioma, neuroblastoma and melanoma.

We are currently investigating (i) the molecular mechanisms by which Kidins220 modulates neuronal excitability, synaptic plasticity, and astrocyte physiology; (ii) the consequences of Kidins220 deletion on plasticity and ageing in the adult mouse brain.

Primary cultures of hippocampal neurons and glial cells are obtained from E18 Kidins220-/- embryos and wild type littermates. To study the role of Kidins220 in the postnatal brain, a conditional knockout mouse line has been generated by crossing the Kidins220-lox line with a transgenic line expressing the Cre recombinase under the Calcium/Calmodulin-dependent protein kinase II (CaMKII) promoter, driving Kidins220 excision only in the excitatory neurons of the forebrain, after postnatal day 14 (Kidins220CaMKII mice).

The effects of Kidins220 deletion manifest themselves more prominently in GABAergic neurons, which are more excitable and recover faster from synaptic depression. Thus, the weight of synaptic inhibition is reinforced, reducing the overall network activity of Kidins220-/- cultures, as shown by multi-electrode array recordings. Besides its well-described role in neurons, Kidins220 plays an important function also in astrocytes, where it modulates BDNF/ proBDNF signalling and Ca2+ transients, thus affecting the efficacy of astrocytes-to-neurons communication. Analysis of Kidins220CaMKII animals revealed defects in brain development, and altered neuron morphology in the motor and somatosensory cortex. The consequences of Kidins220 ablation on the electrophysiological properties of the adult cortico-hippocampal network, and the consequent behavioral outcomes, are currently under investigation.

Altogether, our results extend previous findings on the role of Kidins220 during nervous system development and in embryonic neurons, and identify this protein as an important modulator of neuron and astrocyte physiology, with a prominent role in the shaping of the mature brain circuits of adult animals.

NEUROTROPHIC-PRIMING OF GLUCOCORTICOID ACTIONS

Jeanneteau Fred

Inserm U1191 CNRS UMR5203 Institut de genomique fonctionnelle ~ Montpellier ~ France

Brain disorders comorbid with stress are prevalent worldwide and require therapeutic innovation to reduce the social and economic burden. The problem is that the pathophysiology of these disorders and specific therapeutic targets remains elusive. Two hypothetical frameworks show great promises: the decreased neurotrophic support, and glucocorticoid resistance (decreased responsiveness to glucocorticoids). Yet, the lack of knowledge about the precise sites where glucocorticoids and neurotrophins malfunction has compromised our ability to improve the efficacy and safety of drugs used in treatment modalities. Our published and unpublished data suggest that the glucocorticoid receptor (GR) is a prominent target of the brain-derived neurotrophic factor (BDNF) signaling via GR phosphorylation (℗), which gives rise to unique allostatic changes essential for neuronal adaptation to stress and antidepressant drugs.

Neurotrophic-priming of glucocorticoid function is a promising mechanistic framework to identify novel therapeutic targets and biomarkers of glucocorticoid resistance, a major hallmark of several neuropsychiatric disorders comorbid with stress. The talk will focus on i) describing the mechanism, ii) the physiological role, iii) its deregulation in disease states, and iv) the implications for treating these diseases.

We combined naturalistic stress model, mouse genetics, transcriptomics and in-vivo imaging to examine the role of GR℗ in allostasis to stress.

BDNF signaling can change glucocorticoid receptor responses in vitro and in vivo. The observations ranged from endocrine plasticity of the hypothalamo-pituitary axis to the plasticity of dendritic spines in the motor cortex upon motor learning and stress.

We propose an unifying mechanisms of coincidence detection, which provide new prospects for discovering innovative treatments for disorders featuring impaired BDNF and glucocorticoid activities.

SINGLE MOLECULE IMAGING AND TRACKING OF NEUROTROPHINS AND THEIR RECEPTORS IN LIVING NEURONAL CELLS

Marchetti Laura[1], De Nadai Teresa[2], Amodeo Rosy[1], Bonsignore Fulvio[1], Francesco Gobbo[2], Beltram Fabio[1], Luin Stefano[1], Cattaneo Antonino[2]

[1]NEST, Scuola Normale Superiore and Istituto Nanoscienze – CNR, Piazza San Silvestro 12, I-56126 Pisa, Italy ~ Pisa ~ Italy, [2]BioSNS Laboratory, Scuola Normale Superiore and Istituto di Neuroscienze - CNR, via Moruzzi 1, I-56100 Pisa, Italy ~ Pisa ~ Italy

We currently lack a satisfactory understanding of the membrane complexes and internalization routes underpinning the pleiotropic biological outcomes of neurotrophins (NTs), which exert their functions via interlaced binding of three different families of neurotrophin receptors (NRs).

We are working to answer several open questions in this field: are NRs membrane movements linked to ligand-specific activation processes? Are different receptor functions linked to different movements at the cell membrane? How does p75NTR enhance NGF-TrkA signalling? Are NGF and its precursor proNGF different signalling molecules as far as NRs binding and internalization is concerned?

To address these issues, we developed non-invasive means to covalently fluorolabel with 1:1 stoichiometry both neurotrophins and their receptors. This toolbox was exploited to perform single molecule imaging and tracking (SMIT) at the plasma membrane and inside axons of living neuronal cells using wide-field and TIRF microscopy.

We have so far obtained results in three different directions. First, we gained clues about TrkA membrane mobility and oligomerization state upon binding of four different NTs. We proved that ligand binding shifts TrkA monomer-dimer equilibrium towards the dimer form, and causes the appearance of immobile clustered forms; however the extent of such changes in dynamics is strictly ligand-dependent. We also generated different TrkA mutants allowing for the dissection of three different receptor functions: kinase activity, recruitment of intracellular effectors, ubiquitination and further degradation. Our data point to kinase activity as a master regulator of TrkA membrane dynamics and hint at possible mechanisms by which the cell handles the trafficking of kinase-inactive TrkA receptors. Second, we developed a system for the stable inducible expression of TrkA and p75NTR constructs in living cells, which can be exploited for a dual-color labelling procedure and subsequent simultaneous SMIT of the two receptors. Last, we undertook a comparative study about the axonal transport displayed by “homologue” fluorescent proNGF and NGF in compartmented DRG neurons. We demonstrated that proNGF is internalized and retrogradely transported across axons like mature NGF, but the two NTs display remarkable differences both in terms of NTs flux and number of molecules carried per vesicle. Furthermore, we unveiled a competition mechanism favoring NGF transport upon coadministration of the two NTs.

SMIT analysis is a powerful method to study NTs-NRs membrane dynamics and internalization routes. We are currently optimizing our TIRF setup to get the quantitative description of the kinetics, dynamics and stoichiometry of any molecular complex formed upon proNTs or NTs binding to NRs.

BICD1 AND DYNEIN MODULATE LONG-RANGE GROWTH FACTOR SIGNALLING

Terenzio Marco[1], Golding Matthew[3], Rishal Ida[1], Schiavo Giampietro[2], Fainzilber Mike[1]

[1]Weizmann Institute of Science ~ Rehovot ~ Israel, [2]Institute of Neurology ~ London ~ United Kingdom, [3]Queen Mary University of London ~ London ~ United Kingdom

The development and maturation of extended neuronal networks require tight regulation of the intracellular transport of organelles and cargoes. We focus on dynein-driven retrograde signaling endosomes. The binding fragment of the Tetanus neurotoxin enters these transport compartments, which are shared with neurotrophins and their receptors (Lalli and Schiavo, 2002; Deinhardt et al., 2006). Another component of the dynein-driven retrograde signaling complex is the Bicaudal D family, which comprises BICD1 and BICD2 and two BICD related proteins, BICDR-1 and BICDR-2. BICDs are molecular motor adaptors with pleiotropic roles in intracellular trafficking, which are dependent upon their binding to the molecular motors dynein and kinesin, and to the small GTPase Rab6.

Our goal was to deepen the understanding of the nature of the cellular machinery controlling long-range neurotrophin trafficking in neurons by discovering new players involved in this process through a siRNA screen. Following the candidates, we then concentrated on the role of dynein and dynein adaptors as regulators of long-range trophic factors signaling.

We devised a high-throughput, lipid-based siRNA transfection method in mouse motor neurons derived from ES cells and then used it to screen a library of siRNAs directed against a pool of genes involved in endocytosis and membrane trafficking. Further validation of one of the candidates, BICD1, was performed using standard techniques in molecular and cell biology, including ES cells culture and differentiation in motor neurons, confocal microscopy, surface biotinilation, internalization assays, signaling assays, generation of gene-trapped ES cell lines and embryos, immuno electro-microscopy.

We screened a library of siRNAs directed against a pool of genes involved in endocytosis and membrane trafficking in mouse motor neurons. The primary candidate genes were subsequently validated, and BICD1 was selected for further analyses. Bicd1 expression was found to be restricted to the developing nervous system when neurotrophin receptor expression peaks. Depleting BICD1 increased the intracellular accumulation of BDNF-activated TrkB and p75NTR by disrupting the endosomal sorting, reducing lysosomal degradation and re-routing these active receptors to the plasma membrane. The rebalancing of the population of signaling competent receptors at the neuronal plasma membrane resulted in attenuated, but more sustained, AKT activation. We are currently investigating the role of other members of the dynein complex in relaying long range growth factor signaling.

The data above mentioned suggest that BICD1 is a master regulator of neurotrophin signalling by modulating the endosomal sorting of internalised ligand-activated receptors.

Symposium 14

DYSKINESIA AND NEUROINFLAMMATION: EVIDENCE FROM PULSATILE VERSUS CONTINUOUS L-DOPA DELIVERY

Carta Anna R.[1], Mulas Giovanna[1], Espa Elena[1], Fenu Sandro[1], Lecca Daniela[1], Spiga Saturnino[2]

[1]Dept. Biomedical Sciences, Univ. of Cagliari ~ Cagliari ~ Italy, [2]Dept Life and Environmental Sciences, Univ. of Cagliari ~ Cagliari ~ Italy

Long-term use of L-DOPA in Parkinson disease (PD) is associated with the onset of movement complications, including dyskinesia. Neuroinflammation is a main component of PD neuropathology, and recent evidence suggests that neuroinflammation may also be implicated in the development of L-DOPA-induced dyskinesia (LID).

The present study investigated the contribution of inflammatory responses in the development of LID, by comparing a dyskinetic and a low dyskinetic L-DOPA regimen in the 6-OHDA rat model of PD and in Parkinsonian patients.

Hemiparkinsonian rats were chronically treated with L-DOPA (6 mg/kg/day s.c. for 15 days) in a pulsatile regimen of administration (DOPAp), L-DOPA continuously delivered by osmotic minipumps implanted subcutaneously, (DOPAc 12 mg/kg for 15 days), or DOPAp followed by DOPAc. Moreover, one group of rats was challenged with a single peripheral dose of lipopolysaccharide (LPS, 2 mg/kg i.p.) 24 hrs before pulsatile L-DOPA chronic treatment. Abnormal involuntary movements (AIMs) were evaluated on alternate days during the L-DOPA treatment as an index of dyskinetic responses. At conclusion of all treatments, confocal analysis of OX-42 was performed in the dopamine-depleted dorsal striatum to assess microglia reactivity, and TNF-α-IR was evaluated both in microglia and neurons in the same area. In a pilot study, blood samples were collected from advanced PD patients under oral L-DOPA treatment and experiencing troublesome dyskinesia, or from advanced PD patients under DUODOPA treatment and stabilized motor responses with low dyskinesia.

DOPAp treatment induced a gradual development of AIMs, while treatment with DOPAc did not induce any dyskinetic response in drug naïve rats nor in rats previously rendered dyskinetic by DOPAp dosing. In the dopamine-depleted striatum, DOPAp-induced LID was associated with microglia reactivity, as shown by OX-42-IR, and greater levels of TNF-α colocalization in both microglia and neurons, while DOPAc to drug naïve rats did not induce any inflammatory response. Moreover, reactive microglia void of TNF-α-IR was present after DOPAc treatment in rats, which had previously developed LID by DOPAp. Parkinsonian patients displayed higher levels of TNF-α as compared to healthy control subjects, regardless the L-DOPA treatment received. Preclinical results suggest that microgliosis with increased TNF-α levels were associated with a dyskinetic L-DOPA treatment but not with a non-dyskinetic continuous drug delivery. Clinical results suggest that peripheral TNF-α is increased in PD patients, but does not correlate with presence of troublesome dyskinesia.

Study supported by Perry & Ruby Stevens Charitable Fundation.

APPROACHES TO THE TREATMENT OF L-DOPA INDUCED DYSKINESIA BASED ON RAS-ERK TARGETING: FAR FROM BEING A SIMPLE MATTER

Fasano Stefania

San Raffaele Scientific Institute, Via Olgettina 58 ~ Milan ~ Italy

L-DOPA Induced Dyskinesia (LID) is a common and severe motor complication of current L-DOPA pharmacotherapy of Parkinson's Disease (PD), characterised by the expression of abnormal involuntary movements (AIMs). At the molecular and cellular level, chronic L-DOPA administration results in aberrant dopamine D1-like receptor signalling and in hyperactivation of the Ras-ERK cascade in the striatal medium spiny neurons (dMSNs) of the direct pathway. In addition, large aspiny cholinergic interneurons (ChIs) have been additionally implicated in LID.
Previous work has shown that targeting Ras-ERK signalling may significantly ameliorate AIMs both in rodent and primate models of LID. The use of MEK inhibitors, which specifically block the ERK cascade, has been suggested as a potential pharmacological approach to treat LID. However, these drugs, active on targets involved in diverse cellular processes from cell proliferation/survival to cell differentiation and plasticity, may result in significant side effects and toxicity. We previously shown that targeting of the neuronal specific Ras-GRF1 guanine nucleotide exchange factor, an upstream integrator of both glutamatergic and dopaminergic signals on the ERK cascade in the striatum, is an effective mean to reduce AIMs, also in combination with MEK inhibitors. The observation that ablation of Ras-GRF1 only partially reduces AIMs suggests that other signalling factors may also be implicated in LID.

We have investigated how selective blockade of striatal Ras-GRF1 and its close homolog, Ras-GRF2, impacts on LID (induction, manifestation and maintenance) and its neurochemical correlates.

We used striatal specific viral assisted RNA interference (RNAi) technology to obtain gene knockdown of Ras-GRF1 and Ras-GRF2 in the 6-OHDA mouse model of PD. Escalating doses of L-DOPA were administered and then behavioural analysis with immunohistochemical assays and in vivo microdialysis were performed.

Our findings demonstrate that: 1) ablation of Ras-GRF1 and Ras-GRF2 does not interfere with either basal motor behaviour or motor learning in 6-OHDA mice before L-DOPA treatment; 2) inhibition of Ras-GRF1 but not Ras-GRF2 attenuates dyskinesia development; 3) Ras-GRF1 ablation reduces ERK dependent signalling in the dMSNs but not in ChIs; 4) attenuation of Ras-GRF1 significantly reverts already established dyskinesia.

We confirm that Ras-GRF1 is a promising target for LID therapy based on Ras-ERK signalling inhibition. We also show that ERK signalling in striatal cholinergic interneurons is not dependent on Ras-GRF1 activity.
Therefore, we propose that future development of specific tools to target Ras-ERK in ChIs may further improve the therapeutic efficacy against LID.

DOPAMINE D3 RECEPTOR MODULATES L-DOPA-INDUCED DYSKINESIA BY TARGETING D1 RECEPTOR-MEDIATED STRIATAL SIGNALING

Moratalla Rosario, Garcia--Montes Jose Ruben, Xuan Ming, Solis Oscar

Instituto Cajal, CSIC ~ Madrid ~ Spain

Although the dopamine D3 receptor (D3R) is principally located in the ventral striatum, overexpression of D3R in the dorsal striatum has been reported following L-DOPA treatment in dopamine-denervated animals. However, it is not known the role of D3 receptors in L-DOPA-induced dyskinesia (LID).

To investigate the role of dopamine D3 receptor subtype in L-DOPA-induced dyskinesias and in the changes in gene expression.

Dopamine D3 receptor knockout mice were used to assess the role of D3R in LID and rotational sensitization in the hemiparkinsonian mouse model, as well as the related molecular markers by immunohistochemistry. We also studied the L-DOPA-induced D3R expression in the dorsal striatum of bacterial artificial chromosome transgenic (D1R-tomato fluorescent protein) mice, in order to investigate whether its expression occurs in the D1R or in the D2R medium spiny neurons.

Mice lacking D3R exhibited significantly fewer dyskinetic movements with no change in the antiparkinsonian effect of L-DOPA. However, there was no significant difference in the rotations induced by L-DOPA. Interestingly, deleting D3R attenuated important molecular markers in the D1 striatonigral neurons including FosB, ERK and histone 3 (H3) activation. Colocalization studies indicated that D3R is principally expressed in D1R direct pathway neurons although is also present in the D2R indirect pathway neurons.

Our results demonstrate that the D3R modulates the development of dyskinesia by reducing D1R-mediated intracellular signaling such as FosB, ERK and H3 activation, and suggest that decreasing D3R activity could help to ameliorate LID.

INTENSIVE REHABILITATION IN THE TREATMENT OF L-DOPA-INDUCED DYSKINESIA

Frazzitta Giuseppe

Department of Parkinson Disease and Brain Injury Rehabilitation, "Moriggia Pelascini" Hospital, ~ Gravedona ed Uniti ~ Italy

A major adverse effect of levodopa therapy is the development of dyskinesia, which affects 30–40% of chronically treated Parkinsonian patients.

We hypothesized that an intensive multidisciplinary rehabilitation treatment (MIRT) might allow a reduction in Levodopa dosage without worsening motor performances, thus reducing frequency and severity of dyskinesias.

Ten Parkinsonian patients underwent a 4-week multidisciplinary intensive rehabilitation treatment (MIRT). Patients were evaluated at baseline, at the end of the rehabilitation treatment and at 6 month follow-up. Outcome measures were the Unified Parkinson's Disease Rating Scale Sections II, III, and IV (UPDRS II, III, IV) and the Abnormal Involuntary Movement Scale (AIMS).

At the end of the MIRT, Levodopa dosage was significantly reduced ($p=0.0035$), passing from 1016 ± 327 to 777 ± 333 mg/day. All outcome variables improved significantly ($p<0.0005$ all) by the end of IRT. At follow-up, all variables still maintained better values with respect to admission ($p<0.02$ all). In particular AIMS score improved passing from 11.90 ± 6.5 at admission to 3.10 ± 2.3 at discharge and to 4.20 ± 2.7 at follow-up.

Our results suggest that it is possible to act on dyskinesias in Parkinsonian patients with properly designed rehabilitation protocols. The MIRT, whose acute beneficial effects are maintained over time, might be considered a valid non-invasive therapeutic support for Parkinsonian patients suffering from dyskinesia, allowing a reduction in drugs dosage and related adverse effects.

Symposium 15

THE BCLI RECEPTOR GENE POLYMORPHISM (RS41423247) MODERATES THE ASSOCIATION BETWEEN CHILDHOOD OVERWEIGHT, PSYCHOPATHOLOGY, AND CLINICAL OUTCOMES IN EATING DISORDERS PATIENTS: A 6 YEARS FOLLOW UP STUDY

Ricca Valdo[1], Castellini Giovanni[2], Lorenzo Lelli[2], Tedde Andrea[2], Piaceri Irene[2], Bagnoli Silvia[2], Sorbi Sandro[2], Nacmias Benedetta[2]

[1]Department of Neuroscience, Psychology, Drug Research and Child Health ~ Firenze ~ Italy, [2]Department of Neuroscience, Psychology, Drug Research and Child Health ~ Firenze ~ Italy

Overweight during childhood and the BclI receptor gene polymorphism (rs41423247) of the glucocorticoid receptor gene have been reported to represent predisposing factors for Eating Disorders (EDs).

According to a gene–environment interaction, we tested whether overweight during childhood increased the risk for development of EDs in genetically vulnerable individuals, and whether the BclI receptor gene polymorphism moderated different long term outcomes of EDs.

The distribution of the BclI polymorphism was evaluated in a series of 202 EDs patients referring to the outpatient clinic of Florence (Italy) for EDs, and in 116 healthy subjects. The Structured Clinical Interview for the DSM-IV and self-reported questionnaires were administered at the admission to the clinic and at 3 different time points (end of a cognitive behavioral therapy, 3 and 6 years follow up). Overweight during childhood was assessed by mean of medical records and a clinical interview.

G allele was associated with history of overweight during childhood, depressive disorder comorbidity, and diagnostic instability across time. G allele carriers reporting a history of overweight during childhood showed greater frequency of subjective binge eating episodes and emotional eating, as compared to subjects without this condition, while within patients with CC genotypes this difference was not detected.

The interaction between the BclI polymorphism and overweight during childhood characterized a sub-population of EDs patients with mood disorder comorbidity and a specific diagnostic trajectory in the long term. Furthermore, an early onset of overweight combined with individual vulnerability can determine severe psychopathological consequences in adulthood.

ANIMAL MODELS OF EATING DISORDERS AND OBESITY OR FOOD ADDICTION

Lutz Thomas

University of Zurich ~ Zurich ~ Switzerland

The incidence of obesity and other eating disorders continues to climb worldwide, making it imperative that animal models sharing characteristics of human obesity and its co-morbidities be developed in the quest for novel preventions or treatments.

Eating is controlled by a plethora of factors; most importantly peripheral gastrointestinal hormones that act directly or indirectly on the central nervous system play a major role.

Reward driven processes, which are influenced by gastrointestinal hormones, can overcome the homeostatic controls of eating under certain conditions.

Further, sex differences in the occurrence of some eating disorders may depend on estrogen's modulation of eating controls.

This presentation will summarize some commonly used animal models of obesity and eating disorders like binge eating or anorexia nervosa. The role of estrogen in the control of eating and in eating disorders will also be discussed. Finally, the role of amylin in the control of eating and in the context of neurodegenerative diseases will be summarized.

ROLE OF OLEOYLETHANOLAMIDE IN THE "GUT-TO-BRAIN AXIS": POSSIBLE IMPLICATIONS FOR THE TREATMENT OF OBESITY AND EATING DISORDERS

Romano Adele

Dept. of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome ~ Rome ~ Italy

Current treatments for obesity and eating disorders lack sufficient efficacy and are complicated by high relapse rates and a wide range of side effects, thus highlighting the need to identify novel pharmacological targets for the development of more effective and safer therapies. Among these potential novel targets, we have been focusing on the role played by oleoylethanolamide (OEA), a lipid satiety signal generated in the intestine that appears involved in the "gut to brain axis" controlling food intake. Systemically administered OEA produces a significant inhibition of feeding and the induction of c-fos in key brain areas involved in the control of food intake, such as the nucleus of the solitary tract (NST), the area postrema (AP), and the hypothalamic tuberomammillary (TMN) paraventricular (PVN) and supraoptic nuclei (SON). Different neuronal pathways, including oxytocinergic, noradrenergic, and histaminergic neurons, seem to mediate OEA hypophagic action.

Whether OEA signal can directly or indirectly reach the brain remained to be fully elucidated. To address this issue, we assessed the role of vagal afferent fibers and AP, in OEA-induced behavioral and neurochemical effects in rats.

To this aim, we evaluated the effects of peripherally administered OEA on feeding behavior, and on the activation of brainstem neurons and hypothalamic oxytocin neurons in rats subjected either to a subdiaphragmatic deafferentation (SDA), a surgery that eliminates abdominal vagal afferents but leaves about 50% of the vagal efferents intact, or to a surgical lesion of AP.

The results obtained from SDA experiment demonstrated that the ablation of vagal afferents is not crucial for the anorexiant effect of OEA; in fact, we found that peripherally OEA reduced food intake similarly in SDA and sham-operated rats. Moreover, within the NST of SDA-rats, OEA was still able to increase the expression of c-fos, although this effect was attenuated compared with sham-operated rats. AP lesion prevented both the anorexigenic effects of OEA and the increase of oxytocin expression in PVN and SON, thus suggesting a necessary role of the AP in mediating OEA's effects.

Overall, these findings suggest that OEA signal may reach the brain through a dual mechanism, which involve both afferent vagal fibers and AP. These observations further support the hypothesis that, by modulating food intake, OEA might represent a novel potential pharmacological target for the treatment of hyperphagia and other aberrant eating patterns that contribute to the development of obesity and eating disorders.

ROLE OF THE CENTRAL HISTAMINERGIC SYSTEM IN THE HYPOPHAGIC, PROCOGNITIVE AND ANTIDEPRESSANT-LIKE EFFECTS OF THE ENDOGENOUS PPAR-A LIGAND OLEOYLETHANOLAMIDE

Provensi Gustavo

Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA) – Sezione di Farmacologia e Tossicologia. Universitá di Firenze. ~ Firenze ~ Italy

Oleoylethanolamide (OEA) is an endogenous agonist of the nuclear peroxisome proliferator-activated receptor alpha (PPARα) released by enterocytes in response to fat intake that indirectly signals satiety to hypothalamic nuclei. Using different behavioural settings, we recently reported that the OEA-induced hypophagic effect is significantly attenuated in mice deficient of the histamine-synthesizing enzyme histidine decarboxylase (HDC-KO) or acutely depleted of histamine with i.c.v. infusions of the HDC blocker α-fluoromethylhistidine (α-FMH). Conversely, increased histamine release elicited by the treatment with the H3R antagonist ABT-239 potentiated OEA-induced hypophagia. Moreover, OEA augmented histamine release in the cortex of fasted mice with a time course comparable to its anorexic effects.

These observations led us to investigate whether the interaction between OEA and brain histamine is relevant also for other reported PPARα-mediated effects, namely antidepressive and promnesic actions using behavioural and neurochemical approaches.

Wild type and HDC-KO mice were i.p. treated with vehicle, OEA (5 and 10 mg/kg) or imipramine (10 mg/kg) using two different regimens: sub-chronic (24, 5 and 1 hour before test) and chronic (once daily for 7 days) and then challenged in the tail suspension test (TST), a classical behavioural model predictive of drugs' antidepressant-like effect. OEA-induced procognitive effects were evaluated challenging rats in the contextual fear conditioning paradigm. After training (7 footshocks, 1 mA, at 30s intervals) rats received vehicle or OEA injection (10 mg/kg i.p.) and memory was assessed as 'freezing' time (total absence of movements) 72 hours after injections. Single probe microdialysis was used to investigate OEA-induced changes in histamine release from select brain areas.

Both OEA regimens induced a dose-dependent reduction of immobility time in the TST comparable with the imipramine-induced effect in WT mice, but not in HDC-KO mice. OEA-treated rats showed a longer freezing time as compared to vehicle-treated animals. Infusion of α-FMH into the lateral ventricles or local infusion of either pyrilamine or zolantidine (H1R and H2R antagonists, respectively) into the basolateral amygdala prevented such effect. Accordingly, we observed a fast and transient increase of histamine release from the basolateral amygdala of freely moving rats after OEA i.p. injection at the same dosage used in the behavioural paradigm.

Taken together, these results indicate that the histaminergic system contributes not only to OEA-induced hypophagic effect, but also to other PPARα.-mediated effects such as antidepressant-like and precognitive actions, suggesting that these interactions may be attractive targets for the development of innovative drugs.

Symposium 16

THE NASAL CAVITY AS RAILWAY OF PATHOGENS AND TOXINS TO THE BRAIN

Kristensson Krister[1], Tesoriero Chiara[2], Codita Alina[3], Mingdong Zhang[1]

[1]Department of Neuroscience, Karolinska Institutet ~ Stockholm ~ Sweden, [2]Department of Neurological and Movement Sciences ~ Verona ~ Italy, [3]3Department of Neurobiology, Care Sciences and Society, Section of Neurogeriatrics ~ Huddinge ~ Sweden

A most ill-famed way for a pathogen to enter the brain is that leading from the nasal cavity. The idea of a viral spread along the olfactory route is considered to be one of the most unfortunate in medicine, it delayed polio prophylaxis by several decades and left one generation of Canadian school children anosmic following etching the olfactory epithelium as a preventive attempt. The idea was abandoned and the route fell into almost total neglect. An alarm to the neglect is, however, the brain-eating ameba Naegleria fowleri that thrives in contaminate swimming pools. After inhalation, the ameba can degrade the olfactory epithelium, traverse the cribriform plate and digest the brain parenchyma. In fact, the nasal cavity is unique because neurons in the olfactory epithelium are here in direct contact with the environment. They can pick up protein tracers by fluid phase and lectins by selective adsorptive endocytosis, and therefore bridge molecules in the air with the olfactory bulbs and neuronal populations in their connection. Such nerve cell populations can therefore be targets for transneuronal spread of certain viruses. For instance, measles virus instilled in the nasal cavity can spread into limbic structures of the brain. The targeting can be remarkably selective: 12-day-old mice exposed in the nostrils to droplets of a mutant rabdovirus strain suspension can be left with a selective serotonin deficiency for the rest of their life-span after viral attack on dorsal raphe neurons.

We have now explored the potentials for a H1N1 influenza A virus to attack sleep-wake regulating neurons, which project to the olfactory bulbs.This was done within the context of an increased incidence in the sleep disorder narcolepsy associated with the 2009-2010 pandemic of H1N1 influenza virus.

EEG, EMG, Neck movement, PCR, immunohistochemistry were used for the study.

We show that infection with H1N1 influenza virus in mice, which lack B and T cells (Recombinant activating gene 1-deficient mice), can lead to narcolepsy-like sleep-wake fragmentation and sleep structure alterations. Moreover, the infection targets brain stem and hypothalamic neurons including orexin/hypocretin neurons, which regulate sleep-wake stability and are affected in narcolepsy.

In the light of a recent observation showing binding of seasonal influenza A virus strains to human olfactory epithelium, our findings may be of clinical relevance. A re-awakening of studies on the potentials of seletive viral targeting to neurons projecting to the olfactory system is therefore prompted.

THE ENTERIC NERVOUS SYSTEM AND THE PROGRESSION OF PARKINSON'S DISEASE: FACTS AND PERSPECTIVES

Pizzi Marina[1], Bellucci Arianna[2], Parrella Edoardo[2], Benarese Marina[2], Lanzillotta Annamaria[2], Porrini Vanessa[2], Vegezzi Gaia[2], Liou Hsiou Chi[3], Spano Pier Franco[1]

[1]University of Brescia and IRCCS San Camillo Venice ~ Brescia ~ Italy, [2]University of Brescia ~ Brescia ~ Italy, [3]Cornell University ~ New York ~ United States

Intra-neuronal aggregates of α-synuclein known as Lewy bodies or Lewy neurites are key neuropathological alterations in multiple brain regions in patients with Parkinson's disease (PD). Interestingly, Lewy pathology is also evident in the enteric nervous system (ENS) in PD patients and in some neurologically normal individuals with constipation. Braak and colleagues proposed that α-synuclein pathology is present in the periphery before it reaches the brain and triggers the motor symptoms of PD. They went on to suggest that it spreads from ENS along interconnected neural pathways to reach the dorsal motornucleus of vagus (DMV) as early as 10–20 years before the onset of any motor symptoms. The staging scheme is consistent with the fact that most PD patients have non-motor symptoms, including gastroeneteric dysfunction, that appear much before the motor disorder. This phenomenon might rely on the capability of enteric neurons to release α-synuclein, but how a-synuclein is uptaken by terminals of vagal nerve and spreads to brain remains to be elucidated. To unravel the role of peripheral α-synuclein in the onset and spreading of Lewy body-like pathology as well as neurodegeneration in PD, experimental models reproducing the aging-dependent pathological progress and the progressive non-motor and motor syndrome are essential. We have previously shown that 18 month-old mice lacking NF-kappaB/c-Rel (c-rel-/- mice) develop a dopamine neuronal loss in substantia nigra pars compacta with accumulation of aggregated α-synuclein, microglia activation and specific motor deficits responsive to L-DOPA administration.

Our aim was to investigate whether PD-like pathology in c-rel-/- mice is anticipated by a premotor disorder and α-synuclein accumulation in ENS and lower brainstem.

c-rel -/- and c-rel +/+ mice were investigated for gastric emptying, cholon motility and brain synuclein pathology at 2, 5, 7, 12 months.

We found that at a premotor stage, c-rel-/- mice suffered from gastrointestinal dysfunctions, slower gastric empting and reduced cholon motility. In parallel with the early nonmotor manifestations, at a stage when no synuclein pathology is evident in the SN yet, young c-rel-/- mice accumulate α-synuclein in the DMV and in the locus coeruleus.

Future work should focus on the accumulation and fate of α-synuclein in the DMV. It has to clarify whether α-synuclien derives from enteric neurons and whether and how it triggers brain pathology.

THE OLFACTORY MUCOSA IN THE DIAGNOSIS OF PRION AND PRION-LIKE NEURODEGENERATIVE DISEASES: FACTS AND PERSPECTIVES

Zanusso Gianluigi[2], Caughey Byron[1]

[1]Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health ~ Hamilton, Montana ~ United States, [2]Department of Neurological and Movement Sciences, University of Verona ~ Verona ~ Italy

The involvement of olfactory pathway in different neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's disease (PD), represents a very early event in the disease propagation. In particular, olfactory neurons (ONs) which are located in the nasal vault showed pathological changes found in AD consisting in dystrophic neurites containing of tau paired helical filaments, amyloid-β deposits or alfa-synuclein.

In olfactory mucosa (OM) biopsies from patients with sporadic Creutzfedlt-Jakob disease (sCJD) we demonstrated that prions accumulated in the olfactory epithelium. More recently, we described a much safer and less invasive nasal brushing procedure that allows a gentle collection of OM from a wide surface area of OE, bypassing potential complications of OM biopsies.

We identified prion seeding activity in olfactory mucosa (OM) of patients with sCJD patients by analyzing OM samples with Real Time Quaking induced Conversion (RT-QuIC), an ultrasensitive, multi-well plate-based fluorescence assay involving pathological PrP seeded polymerization of recombinant PrP into amyloid fibrils.

We obtained a 100% specificity and >97% sensitivity for the detection of CJD. Other misfolding diseases such as AD and PD involve aggregation of their respective misfolded proteins (e.g., Aβ, tau or α-synuclein) and the mechanisms of replication is of these proteins is prion-like.

Here, we show our progress in developing a highly sensitive seeding assays of OM brushings, analogous to our prion test, addressed to provide an intravital diagnosis of other brain proteinopathies.

Symposium 17

CRITICAL ROLE OF D-SERINE IN SYNAPTIC PLASTICITY RELEVANT TO COCAINE ADDICTION

Marcello D'Ascenzo

Università Cattolica del Sacro Cuore ~ Rome ~ Italy

D-serine, a D-amino acid that has been found at high levels in mammalian brain, binds with high affinity the co-agonist site of N-methyl-D-aspartate receptor (NMDAR) and mediates, along with glutamate, several important processes including synaptic plasticity.

Aim of the present study was to investigate the possible involvement of D-serine signaling in cocaine-induced impairment of synaptic plasticity at excitatory synapses in the nucleus accumbens (NAc), a brain region that has a central role in the development and expression of addictive behaviors.

To this aim we performed electrophysiological and molecular biology experiments on medium spiny neurons of the NAc of control and cocaine-treated rats. Furthermore, in vivo experiments on rats were performed to evaluate the impact of D-serine signaling in cocaine-induced locomotor sensitization.

We found that D-serine is the endogenous coagonist of NMDAR in the NAc, where its presence is essential for NMDAR-dependent long-term potentiation (LTP) and depression (LTD). Interestingly, we also provide novel evidence that NAc slices obtained from cocaine-treated rats after 1 day of abstinence presented significantly reduced D-serine concentrations, increased expression of the D-serine degrading enzyme, D-amino acid oxidase, and downregulated expression of serine racemase, the enzyme responsible for D-serine synthesis. The D-serine deficit was associated with impairment of LTP and LTD that was restored by slice perfusion with exogenous D-serine. In addition, we found that in vivo microinjection of D-serine directly into the NAc blocks behavioral sensitization to cocaine.

We propose a new role for D-serine signaling as molecular correlate for cocaine-induced changes in synaptic plasticity and locomotor sensitization.

D-ASPARTATE-BASED MODULATION OF NMDA RECEPTOR NEUROTRANSMISSION: PRECLINICAL IMPLICATION

Errico Francesco[1], Sforazzini Francesco[2], Squillace Marta[1], Napolitano Francesco[1], Angrisano Tiziana[3], Di Maio Anna[1], Vitucci Daniela[1], Chiariotti Lorenzo[3], Gozzi Alessandro[2], Usiello Alessandro[1]

[1]Ceinge Biotecnologie Avanzate ~ Naples ~ Italy, [2]Istituto Italiano di Tecnologia, Center for Neuroscience and Cognitive Systems ~ Rovereto ~ Italy, [3]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II" ~ Naples ~ Italy

Increasing evidence points to a role for dysfunctional N-methyl-D-aspartate receptor (NMDAR) neurotransmission in schizophrenia. D-aspartate is an atypical amino acid that activates NMDARs through the binding to their glutamate site. D-aspartate occurs abundantly in the embryonic brain of mammals and rapidly decreases after birth, due to the activity of the enzyme D-Aspartate Oxidase (DDO).

The agonistic activity of D-aspartate on NMDARs and its neurodevelopmental occurrence make this D-amino acid a potential mediator for some of the NMDAR-related alterations observed in schizophrenia. Consistently, substantial reduction of D-aspartate was recently observed in post-mortem frontal samples of schizophrenic patients.

We evaluated DDO mRNA expression and degree of methylation in the putative promoter region of DDO gene in the post-mortem prefrontal cortex of schizophrenic patients. Moreover, we treated knockout mice for Ddo gene (Ddo-/-) with the NMDAR antagonist phencyclidine to evaluate their schizophrenia-relevant behaviors and circuits. Finally, we also assessed cortico-hippocampal connectivity of these mice.

We found that DDO mRNA expression is increased in frontal samples of schizophrenic patients, thus suggesting a plausible molecular event responsible for the D-aspartate imbalance previously described. Moreover, we revealed that Ddo-/- mice display a significant reduction in motor hyper-activity and prepulse inhibition deficit induced by phencyclidine. Furthermore, increased levels of D-aspartate in Ddo-/- animals can significantly inhibit functional circuits activated by phencyclidine, and affect the development of cortico-hippocampal connectivity networks potentially involved in schizophrenia.

Our data highlight a potential contribution of D-aspartate as putative key neurodevelopmental modulator of brain circuits and behaviors relevant to schizophrenia.

OF THE IMPORTANCE OF SPACE AND TIME IN THE FUNCTIONS OF THE NMDA RECEPTOR COAGONISTS IN THE HIPPOCAMPUS

Mothet Jean Pierre

CNRS - Aix Marseille University ~ Marseille ~ France

NMDA receptors (NMDARs) support patterning and activity of synapses throughout life and are central to many brain disorders. The NMDAR activation requires the concomitant binding of glutamate and a coagonist glycine or D-serine. To date, whether a preference for one coagonist at specific connections occurs remains unsolved.

Here, we sought to determine when and where D-serine and glycine enter into play at hippocampal synapses to regulate synaptic NMDA receptors and whether their functions are exclusive or not at those synapses during basal synaptic transmission and during high levels of synaptic activity.

We investigated the contribution of D-serine and glycine by recording the NMDAR-mediated field excitatory postsynaptic potentials at hippocampal Schaffer collaterals (SC)-CA1 and medial perforant path-dentate gyrus (mPP-DG) synapses in juvenile and adult rats. Functions of D-serine and glycine during synaptic transmission and synaptic plasticity were probed by selective depletion of endogenous coagonists with enzymatic scavengers as well as pharmacological inhibition of endogenous D-amino acid oxidase activity and of glycine transporters (Glyt1). Furthermore, we have investigated the presence of specific NMDARs subunits using pharmacological intervention and biochemical assays.

We revealed that D-serine is the preferred coagonist at SC–CA1 mature synapses, whereas, unexpectedly, glycine is mainly involved at mPP–DG synapses. Nevertheless, both coagonist functions are driven by the levels of synaptic activity as inferred by recording long-term potentiation generated at both connections. This regional compartmentalization in the coagonist identity is associated to different GluN1/GluN2A to GluN1/GluN2B subunit composition of synaptic NMDARs. During postnatal development, the replacement of GluN2B- by GluN2A-containing NMDARs at SC–CA1 synapses parallels a change in the identity of the coagonist from glycine to D-serine. In contrast, NMDARs subunit composition at mPP–DG synapses is not altered and glycine remains the main coagonist throughout postnatal development. Remarkably, this segregation coincides with the subunit composition of postsynaptic NMDARs and the maturation of the tripartite synapse.

Our observations disclose an unprecedented relationship in the identity of the coagonist not only with the GluN2 subunit composition at synaptic NMDARs but also with astrocyte activity in the developing and mature hippocampus that reconciles the complementary functions of D-serine and glycine in modulating NMDARs during the maturation of tripartite glutamatergic synapses.

D-SERINE METABOLISM IN THE BRAIN: THE ROLE OF D-AMINO ACID OXIDASE

Sacchi Silvia, Cappelletti Pamela, Caldinelli Laura, Molla Gianluca, Pollegioni Loredano

Dipartimento di Biotecnologie e Scienze della Vita, Università degli Studi dell'Insubria ~ Varese ~ Italy

Despite their atypical nature, it is now widely assumed that free D-amino acids are active molecules at synapses capable of modulating synaptic communication within neuronal networks in mammals. D-Serine in particular, was reported to influence the functional plasticity of cerebral circuitry by acting as the main coagonist of the N-methyl-D-aspartate receptor (NMDAr) in different brain areas. Accordingly, abnormal D-serine-dependent NMDAr activation has been involved in several neurological pathologies ranging from acute and chronic degenerative diseases (ALS and Alzheimer's disease) to psychiatric disorders, such as schizophrenia and bipolar disorder.

It was demonstrated that an imbalance in the level/activity of the enzymes involved in D-serine metabolism is likely entailed in the onset of such disorders. Among these, the FAD-dependent flavoenzyme D-amino acid oxidase (DAAO), which catalyzes the oxidative deamination of D-amino acids, has been proposed to play an essential role in the catabolism of D-serine. Here, the modulation of human DAAO enzymatic activity will be presented with the final aim to highlight DAAO significance as a therapeutic target in the treatment of different neurodegenerative and neurodevelopmental pathologies.

Amino acid substitutions in hDAAO sequence were introduced by site-directed mutagenesis. The alterations in hDAAO properties induced by the introduced substitutions were investigated by spectroscopic and chromatographic techniques. The effect exerted by chemicals compounds and interacting proteins on the enzyme functionality was evaluated by using specific activity assays. The mechanisms involved in hDAAO inhibition were defined by structural analysis and molecular modeling, whenever possible.

hDAAO shows peculiar biochemical properties, i.e. a weak FAD binding, a low specific activity and a stable dimeric state. The enzymatic activity is finely modulated by the presence of mutations, by different classes of inhibitors and by the interacting protein pLG72. Here we provide new insight into the molecular mechanism by which this occurs.

Here we report on extensive studies concerning the modulation of the enzyme activity of hDAAO, in physiological and pathological conditions. The results demonstrate that hDAAO plays a pivotal role in the regulation of D-serine cellular levels and provide crucial information to the design of more effective hDAAO inhibitors that might be used as drugs to regulate NMDAr activation state by affecting D-serine concentration in the brain. This with the ambitious goal to promote novel therapeutic approaches to target schizophrenia and other disorders in which NMDA receptor mediated neurotransmission is dysregulated.

Symposium 18

DYNAMIC EPIGENETIC REGULATION OF NCX1 EXPRESSION IN BRAIN ISCHEMIA AND IN ISCHEMIC BRAIN PRECONDITIONING, BY MODIFICATION OF HISTONE ACETYLATION

Formisano Luigi[1], Guida Natascia[2], Valsecchi Valeria[1], Cantile Maria[1], Cuomo Ornella[1], Vinciguerra Antonio[1], Laudati Giusy[1], Pignataro Giuseppe[1], Sirabella Rossana[1], Di Renzo Gianfranco[1], Annunziato Lucio[1]

[1]Federico II Univ of Naples ~ Naples ~ Italy, [2]IRCSS, SDN ~ Naples ~ Italy

The Na+-Ca2+ exchanger 1 (NCX1) is a ubiquitous plasma membrane protein regulating cellular calcium and sodium homeostasis and is highly involved in the progression of cerebral ischemia. NCX1 is reduced in stroke by the RE1-silencing transcription factor (REST), whereas it is increased in ischemic brain preconditioning (PC) by Hypoxia-Inducible Factor 1 (HIF-1). Interestingly, ncx1 brain promoter (ncx1-Br) has five putative consensus sequences, named Sp1 A-E, for the Specificity protein (Sp) family of transcription factors (Sp1, Sp2, Sp3, Sp4).

Here, we investigated: (1) the role of Sp family in regulating ncx1 transcription in brain; (2) the presence of two different multiprotein complexes that binding ncx1-Br regulate its expression in in vitro and in vivo models of stroke and PC; (3) the role of Histone-Deacetylaae (HDAC) class I inhibitor MS-275 and Histone-Acetyltransferase (HAT) inhibitor C-646 in in vitro model of stroke and PC on cell survival and its correlation with NCX1 expression.

All in vitro experiments were performed in rat cortical neurons (DIV 7). Brain ischemia was obtained in vivo by transient middle cerebral artery occlusion (tMCAO) and in vitro by Oxygen and Glucose Deprivation plus Reoxygenation (OGD/Rx). EMSA, ChIP and Re-ChIP were used to identify the binding of transcription factors and cofactors on ncx1-Br. Luciferase assay was used to evaluate the activity of the ncx1-Br. q-RT-PCR and Western Blot to study the change in mRNA and protein expression. LDH was used to study neuronal death.

We found that Sp1 is a transcriptional activator, whereas Sp3 is a transcriptional repressor of ncx1, and that both bind ncx1-Br in a sequence-specific manner, modulating ncx1 transcription through the Sp1 sites C-E. Furthermore, in tMCAO the transcriptional repressors Sp3 and REST colocalized with HDAC1 and HDAC2 at the ncx1-Br, with a consequent hypoacetylation. By contrast, in PC+tMCAO the transcriptional activators Sp1 and HIF-1 colocalized with the HAT p300 on ncx1-Br with a consequent hyperacetylation. In addition, in neurons silenced with siRNA of NCX1, and subjected to OGD/Rx, the protective effect of class I HDAC inhibitor MS-275 was counteracted, whereas in neurons overexpressing NCX1 and subjected to PC+OGD/Rx, the neurotoxic effect of p300 inhibitor C646 was prevented.

Collectively, these results demonstrate that NCX1 expression is regulated by Sp3/REST/HDAC1/HDAC2 complex in tMCAO and by the Sp1/HIF-1/p300 complex in PC+tMCAO and that epigenetic intervention, by modulating the acetylation of ncx1 gene promoter may be a strategy for the development of innovative therapeutic intervention in stroke.

ROLE OF PARP AND HDACS IN HIPPOCAMPAL SLICE MODELS OF ISCHEMIC PRECONDITIONING IN VITRO

Pellegrini--Giampietro Domenico[1], Gerace Elisabetta[1], Landucci Elisa[1], Scartabelli Tania[1], Moroni Flavio[2], Chiarugi Alberto[1]

[1]Department of Health Sciences, Clinical Pharmacology and Oncology Unit, University of Florence ~ Firenze ~ Italy, [2]Department of Neuroscience, Psychology, Drug Research and Child Health (NeuroFarBa), University of Florence ~ Firenze ~ Italy

Ischemic tolerance is an evolutionary conserved cellular defense program in which exposure to a subtoxic preconditioning insult results in resistance to a subsequent lethal stressor. Understanding the nature of the endogenous adaptive neuroprotective responses that are elicited by preconditioning stimuli can be important for the development of new theraputic strategies.

The aim of our study was to evaluate the role of poly(ADP-ribose) polymerase (PARP) and histone deacetylases (HDACs) in newly developed pharmacological in vitro preconditioning models.

We used organotypic hippocampal slices exposed to 30 min oxygen-glucose deprivation (OGD), which leads to selective injury of the CA1 subregion 24 h later. We developed our model by exposing the slices to brief bouts of NMDA or DHPG and then, 24 h later, to 30 min OGD.

Under these conditions, we observed a significative reduction in OGD-induced CA1 damage. Exposure of slices to the PARP inhibitors PJ-34 and TIQ-A or to the HDACs inhibitors SAHA and sodium butyrate, prevented the development of OGD tolerance in a dose-dependent manner. To evaluate the activity of PARP in our preconditioning models we carried out western blotting using antibodies against the poly(ADP-ribose) (PAR) polymer product and against the histone H3 acetylated in lysine (K) 18. Our results show that the formation of PAR was increased by preconditioning doses of NMDA in a TIQ-A-dependent manner. Futhermore, 3 h after the preconditioning stimulus we observed a slight increase in H3 (K18) acetylation as compared with the dramatic increase induced by the HDAC inhibitor SAHA. We also observed that the increase in H3 (K18) acetylation induced by NMDA preconditioning was significantly prevented by TIQ-A, whereas the increase in PAR formation was similarly prevented by SAHA, thus suggesting a possible interaction between PARP and HDAC activities in the development of ischemic tolerance. Finally, both TIQ-A and SAHA were able to prevent the increase in pERK 1/2 induced by NMDA preconditioning.

Our results suggest that the interplay between histone acetylation and PARP activity may play an important role in the development of ischemic tolerance.

EPIGENETIC AND TRANSCRIPTIONAL DYSFUNCTION IN ISCHEMIC BRAIN INJURY AND HUNTINGTON'S DISEASE: FROM MECHANISMS TO THERAPEUTIC CANDIDATES

Yildirim Ferah[1], W Ng Christopher[5], A. Gipson Theresa[5], Vashishtha Malini[3], Harms Christoph[2], Meisel Andreas[2], Thompson Leslie M.[3], Housman David E.[5], Fraenkel Ernest[4]

[1]NeuroCure Clinical Research Center, [2]Center for Stroke Research Berlin, Charité - Universitätsmedizin Berlin, Berlin, Germany, [3]Department of Psychiatry and Human Behavior and UCI Institute of Memory Impairments and Neurological Disorders, University of California, Irvine, CA 92697, USA, [4]Department of Biological Engineering, [5]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Aberrant changes in gene expression patterns along with impaired epigenetic regulation play pivotal roles in the pathogenesis of central nervous system diseases. In line, strategies that target epigenetic and gene expression dysfunction such as histone deacetylase (HDAC) inhibition lead to robust beneficial effects in numerous neurological and psychiatric conditions.

Our goal is to evaluate the epigenetic and gene expression changes in neurons in disease to identify the key pathogenic changes in regulation of gene expression. These processes are to become candidates for novel therapeutic interventions, potentially for a range of neurological and psychiatric diseases.

We use a combination of genome-wide DNA sequencing technologies, e.g. chromatin immunoprecipitation (ChIP-Seq) to assess the status of histone modifications and RNA sequencing (RNA-Seq) to measure transcription throughout the genome, together with molecular biology techniques and appropriate cell and mouse models of disease in our studies.

We observed impaired neuronal histone acetylation levels in brain ischemia models and identified cAMP-response element binding protein (CREB)–binding protein (CBP) as a crucial factor in the susceptibility of neurons to ischemic stress. In accordance with our previous results that show neuroprotection by histone acetylation enhancement induced by HDAC inhibition, in the present study, ischemic preconditioning increased histone acetylation levels globally as well as at specific neuroprotective gene promoters extending the role of epigenetic regulation also to endogenous neuroprotection programs. In Huntington’s disease (HD), genome-wide analysis of transcription and histone H3K4me3, an active epigenetic mark, revealed highly coordinated chromatin changes along with the detected transcriptional failure in the affected brain areas in R6/2 mice. Targeting the levels of a H3K4-specific histone demethylase in in vitro HD models rescued down-regulation of key neuronal genes caused by mutant Huntingtin expression and proved protective in a Drosophila HD model.

Our work on brain ischemia suggests that histone acetylation and its machinery, e.g. CBP, determine stroke outcome and play crucial roles for the induction of ischemia-resistance in neurons. Our Huntington’s disease studies identified H3K4me3 as a novel therapeutic target for ameliorating the disease progression in HD. Altogether, our findings show that the epigenome and transcription change in coordination in disease and the epigenetic processes provide unprecedented therapeutic opportunities for rescuing the transcriptional demise thereby achieving protection in ischemic brain injury, Huntington’s disease and potentially in other central nervous system disorders.

Symposium 19

ROLE OF PERINEURONAL NETS IN CNS PLASTICITY AND REPAIR

Faralli Alessio[1], Foscarin Simona[1], Pajaj Ermira[1], Dagna Federico[1], Bekku Yoko[2], Oohashi Toshitaka[2], Albera Roberto[1], Rossi Ferdinando[1], Carulli Daniela[1]

[1]University of Turin ~ Turin ~ Italy, [2]Okayama University ~ Okayama ~ Japan

Structural plasticity of neural circuits depends on the balance between intrinsic neuronal properties and regulatory cues present in the surrounding microenvironment. Among extrinsic plasticity-inhibitors are perineuronal nets (PNNs), which are meshworks of extracellular matrix that enwrap many types of neuron in the adult CNS. Plasticity is also strongly influenced by experience, but it is still unclear how external stimuli modulate PNNs.

To tackle this issue, we asked whether: i) environmental stimulation promotes neuronal plasticity by modifying the expression of PNNs in the cerebellum; ii) vestibular compensation, namely the resolution of motor deficits resulting from a unilateral peripheral vestibular lesion, is accompanied by changes in PNN expression in the deafferented vestibular nuclei.

We examined the effects of an enriched environment (EE) on neuritic remodeling and modulation of PNNs in the cerebellar nuclei of adult mice by immunohistochemistry, morphometric analysis, real-time PCR and in situ zymography. To address whether PNN changes are functional to repair after injury, we performed histochemical analysis of PNN expression and morphological evaluation of axon remodelling in the vestibular nuclei of the adult mouse during vestibular compensation after unilateral labyrinthectomy.

We found that exposure of adult mice to EE for one month induces significant morphological changes of Purkinje and precerebellar axon terminals in the cerebellar nuclei, accompanied by a conspicuous reduction of PNNs. Upon EE, cerebellar nuclear neurons show decreased expression of mRNAs coding for key matrix components and enhanced activity of matrix degrading enzymes. Therefore, external stimuli may act by shifting the balance between synthesis and removal of matrix components in order to facilitate neuritic growth by locally dampening the activity of inhibitory cues.
After unilateral labyrinthectomy, mice show severe vestibular deficits, which gradually recover with time. During the process of vestibular compensation, PNN number and staining intensity are strongly attenuated in the lateral vestibular nucleus on both sides, in parallel with remodelling of excitatory and inhibitory afferents. Moreover, PNNs are completely restored when vestibular deficits are abated. Interestingly, in mice with genetically reduced PNNs, vestibular compensation is accelerated. These results strongly suggest that temporal tuning of PNN expression may be crucial for vestibular compensation.

We can conclude that PNNs are highly modifiable structures, which can be actively modulated to facilitate or restrict structural plasticity according to specific functional requirements. Elucidating mechanisms underlying PNN modulation may have important implications in view of clinical strategies to enhance functional recovery after nervous system injury.

ISCHEMIC AND PHARMACOLOGICAL PRE-CONDITIONING AND POST-CONDITONING: MOLECULAR MECHANISMS OF NEUROPROTECTION AND NEUROPLASTICITY

Gerace Elisabetta[1], Zianni Elisa[2], Gardoni Fabrizio[2], Landucci Elisa[1], Scartabelli Tania[1], Moroni Flavio[3], Mannaioni Guido[3], Di Luca Monica[2], Pellegrini--Giampietro Domenico[1]

[1]1Department of Health Sciences, Clinical Pharmacology and Oncology Unit, University of Florence ~ Florence, ~ Italy, [2]3Department of Pharmacological Sciences, University of Milan ~ Milan ~ Italy, [3]2Department of Neuroscience, Psychology, Drug Research and Child Health (NeuroFarBa), University of Florence ~ Florence, ~ Italy

Ischemic tolerance is an endogenous neuroprotective mechanism by which neurons exposed to a mild preconditioning stress results in resistance to a subsequent lethal ischemic insult, but the underlying mechanisms are poorly understood.

Because cerebral ischemia involves the activation of glutamate receptors, we investigated whether AMPA responses were modified following preconditioning in rat organotypic hippocampal slices, and the possible underlying mechanisms for these changes.

Preconditioning was investigated by exposing rat organotypic hippocampal slices to sublethal concentrations of the mGluR1/5 agonist DHPG (10 µM for 30 min) or NMDA (3 µM for 60 min) and 24 h later, to toxic conditions (30 min OGD or 10 µM AMPA for 24 h). Whole-cell voltage-clamp recordings were used to measure the AMPA-induced EPSCs and Western blot analysis performed in postsynaptic densities were used to measure the expression of the GluA1, GluN2A and GluN2B subunits and of PSD-95. Postconditioning was investigated by exposing organotypic hippocampal slices to 30 min OGD, and 5 min later to 10 µM DHPG for 30 min.

NMDA preconditioning and DHPG pre- and postconditioning significantly reduced the CA1 damage induced by 30 min OGD or AMPA. Twenty-four h after exposure to DHPG or NMDA preconditioning, AMPA induced-currents were significantly reduced and following NMDA, but not DHPG preconditioning, the expression of GluA1 was significantly reduced. This reduction appeared to be associated with the internalization of AMPA receptors in the postsynaptic densities. The CB1 antagonist AM251 prevented the development of tolerance to AMPA toxicity induced by DHPG but not by NMDA. Accordingly, an increase in 2-arachidonoylglycerol (2-AG), but not in anandamide, was also able to induce tolerance.

Preconditioning with NMDA and DHPG induced ischemic tolerance with differential mechanisms: NMDA by internalization of GluA1-AMPA receptors, DHPG by increasing 2-AG content.

ROBOTIC REHABILITATION AND PLASTICIZING TREATMENTS FOR THE RECOVERY OF MOTOR FUNCTION AFTER FOCAL CORTICAL STROKE

Lai Stefano[1], Spalletti Cristina[2], Alia Claudia[3], Panarese Alessandro[1], Ghionzoli Alessio[1], Micera Silvestro[1], Caleo Matteo[2]

[1]Scuola Superiore Sant'Anna, Translational Neural Engineering Laboratory, The BioRobotics Institute ~ Pisa ~ Italy, [2]CNR, Neuroscience Institute ~ Pisa ~ Italy, [3]Scuola Normale Superiore ~ Pisa ~ Italy

Stroke is one of the leading causes of chronic motor disabilities and re-acquisition of motor skills is crucial for stroke survivors. A better understanding of the mechanisms underlying spontaneous and training-induced post-stroke recovery may indicate novel targets for more effective therapeutic strategies.

We investigate the effect of the combination of robot-assisted motor training and plasticizing treatments to rehabilitate motor function in a mouse model of focal stroke.

We developed a semi automatic tool to track the whole kinematics of the paw during a skilled reaching task. In combination with other traditional behavioural tests (i.e Gridwalk and Schallert Cylinder), we used this tool to assess the forelimb motor performance before and after a local ischemic stroke. Endothelin-1 or Photothrombosis were used to induce this unilateral lesion in Caudal Forelimb Area, previously identified and mapped by means of an Intra-cortical micro-stimulation study. We followed the time course of the motor performance, during the sub-acute post-injury period, in several conditions: spontaneous evolution and in combination with a daily robotic training or/and plasticizing treatment (i.e. silencing of the healthy hemisphere through botulinum neurotoxin delivery). For robot-assisted therapy, we developed and validated a robotic platform to train animals in a retraction task of their forelimb, mimicking a quantitative and reliable rehabilitation protocol.

The traditional behavioral tests indicated that severe impairments of contralesional forelimb movement persisted even after 30 days post-stroke. Such deficits were also confirmed by altered kinematics of paw trajectories in the skilled reaching task. The robotic training was effective in improving performance on the retraction task, but failed to produce effects in general motor tests. However, combining robot-assisted therapy with transient synaptic silencing of the healthy side induced substantial recovery of motor function and restoration of original movement patterns.

The silencing of the contralesional hemisphere associated to a robotic rehabilitation training favours plastic cortical reorganization and post-stroke recovery, thus indicating a line along which novel therapeutic strategies could be developed.

MESENCHYMAL STROMAL CELL THERAPY TO FOSTER ENDOGENOUS REPARATIVE MECHANISMS IN THE ACUTE INJURED BRAIN

Pischiutta Francesca[1], Sammali Eliana[3], De Simoni Maria Grazia[1], Parolini Ornella[2], Zanier Elisa R[1]

[1]1IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Department of Neuroscience ~ Milano ~ Italy, [2]3Fondazione Poliambulanza, Centro di Ricerca E. Menni ~ Brescia ~ Italy, [3]Fondazione IRCCS Istituto Neurologico Carlo Besta ~ Milano ~ Italy

Traumatic brain injury and stroke are leading causes of mortality and disability in high income countries. In addition to neurological damage, acute brain injury induces a series of neurorestorative events. Harnessing this restorative capacity in order to repopulate and repair the injured brain following acute injury has become an important therapeutic strategy. Mesenchymal stromal cells (MSC) are ideal candidate since they act on multiple mechanisms of protection and repair by pleiotropic actions including secretion of neurotrophic factors, promotion of angiogenesis and neurogenesis, synaptic plasticity and modulation of the immune response.

To discuss the therapeutic potential of human MSC in stroke and TBI mice with respect to: - early changes in the microenvironment promoting long lasting mechanisms of protection and repair – definition of a preclinical protocol translatable to the clinical setting.

Models: C57BL6/J (young: 2-3 months; aged: 12-15 months) mice were subjected to transient middle cerebral artery occlusion or controlled cortical impact to mimic stroke and TBI, respectively. MSC sources: cord-blood, bone marrow, adipose or amniotic tissue derived MSC were tested. Delivery: local (intracerebroventricular) and systemic (intravenous) administrations were used. Outcome measurements: sensorimotor and cognitive deficits, evaluated longitudinally; post mortem analysis included histological (anatomical damage, vessel density, gliotic scar formation, microglia activation and polarization, neurogenesis and regeneration) and biomolecular (inflammatory genes, microglia/macrophages polarization, growth factors) assessments.

Human MSC: 1) induce an early and persistent improvement of functional and anatomical damage; 2) switch the brain microenvironment from a “hostile” to an “instructive” status promoting healing and regeneration; 3) induce protective changes even in the absence of the cell-cell contact; 4) induce a comparable long term protection when injected systemically or locally in young TBI mice, however in aged mice they are effective only when locally administered.

MSC are able to reprogram the local microenvironment from detrimental to beneficial, favoring protective/pro-regenerative changes of the lesioned tissue and contributing to long term improvement of neurological function. Young and aged mice display important differences in terms of degree of recovery and involved mechanisms after MSC treatment. This is a major issue that needs to be fully investigated since aging is a risk factor for stroke and TBI incidence.

Symposium 20

ROLE OF EXOSOMES RELEASED BY ASTROCYTES OVEREXPRESSING MUTANT SOD1 IN DISEASE SPREADING AND MOTOR NEURON PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

Basso Manuela[1], Bonetto Valentina[2]

[1]Laboratory of Transcriptional Neurobiology - CIBIO, University of Trento ~ Trento ~ Italy, [2]Dulbecco Telethon Institute, IRCCS-Istituto di Ricerche Farmacologiche ~ Milano ~ Italy

In numerous neurodegenerative diseases the interplay between neurons and glia modulates the outcome and progression of the pathology. A particularly intriguing way of interaction between neurons, astrocytes and microglia is characterized by the release of microvesicles able to transport proteins, lipids and nucleotides from one cell to the other.

To examine if astrocytes carrying a pathogenic mutation can selectively induce neuronal death through microvesicles and their content.

We analyzed the ability of astrocytes, expressing mutant copper-zinc superoxide dismutase (SOD1) as a model of Amyotrophic Lateral Sclerosis (ALS), to induce damage in wild type neurons through the transport of toxic material in microvesicles. We characterized the proteomic content of the astrocyte-derived exosomes along with some preliminary studies on their RNA.

We showed that exosomes derived from mutant SOD1-primary astrocyte cultures are sufficient to induce selective motoneuronal death. Accordingly, the protein causative of disease, mutant SOD1, was detected in astrocyte-derived microvesicles and observed as able to fuse into neurons and trigger the pathology in vitro.

We can deduce that astrocytes expressing mutant SOD1 contribute to ALS also by the release of microvesicles containing toxic components. The ability to decipher the molecules that are the major contributors of the pathogenic mechanisms in neurons can offer novel diagnostic opportunities.

EXTRACELLULAR VESICLES ISOLATED FROM BONE MARROW MESENCHYMAL STEM CELLS DISPLAY AN ANTI-INFLAMMATORY EFFECT IN EXPERIMENTAL MODELS OF ALZHEIMER DISEASE

Coco Silvia[3], Elia Chiara[2], Matteoli Michela[1]

[1]CNR- Institute of Neuroscience ~ Milano ~ Italy, [2]University of Milano, Dept. Pharmacological and Biomolecular Sciences ~ Milano ~ Italy, [3]University of Milano Bicocca, Dept. Health Sciences ~ Monza, MB ~ Italy

Mesenchymal Stem Cells (MSCs) are undifferentiated cells that are present in many tissues of the body. They represent a promising therapeutic strategy for cardiovascular, autoimmune and neurodegenerative diseases. It has been found that MSCs have potent immunosuppressive capacity and possess therapeutic potential for various inflammation-related diseases. MSCs modulate the activity of immune system cells, inhibiting the acquisition of a pro-inflammatory phenotype and favouring instead anti-inflammatory actions. This effect is attributable mainly to MSC paracrine activity, consisting in the release of bioactive soluble factors and/or microvesicles (MVs), which are thought to be able to directly activate target cells and modulate their activity, by triggering a protective mechanism.
MSCs in resting state or upon activation, in fact, are able to release MVs that are involved in the regulation and modulation of many physiological processes. MVs can influence cell behaviour through direct contact with target cells, fusion with the cell membrane and/or horizontal transfer of genetic material (such as mRNAs and miRNAs).

In order to investigate the possible therapeutic effect of MSC-derived MVs in neurodegenerative processes, we investigated MV immunomodulatory activity in an in vitro system that recapitulates at least in part the inflammatory environment present in AD pathology.

MSCs were extracted from the bone marrow of C57Bl/6 mice and characterized, by FACS analysis, for stemness markers (SCA1+ and CD73+) and LIN-.
MSC-derived MVs were isolated by ultracentrifugation following a protocol widely recognized by the scientific community and characterized by flow cytometry for the evaluation of CD9, CD29, CD49 antigens expressed on their membranes.
MVs were then incubated with primary microglial cells previously exposed to hAbeta 1-42 peptide. MV immunomodulatory capacity was assessed at different time points by evaluating, through ELISA, FACS, RT-PCR and immunocytochemistry, the expression of specific markers or the release of cytokines.

In the presence of MVs, microglial cells assume a M2-like phenotype, which reflect proregenerative and immunoregulatory functions. This is paralleled by an increased release of anti-inflammatory cytokines (IL10), without significant changes in the release of the pro-inflammatory TNFalpha and IL6. Consistently, MVs lead to the decrease in the expression of markers like MHC II, associated with a pro-inflammatory phenotype. In order to find out whether this effect may have a positive impact in vivo, we are presently running experiments in AD mice models.

Altogether these results support an anti-inflammatory role of MVs in vitro. The involved molecular mechanisms are under investigation.

SHUTTLING OF MICRORNAS FROM GLIA-TO-NEURONS: MODULATION OF NEURONAL GENE EXPRESSION

Prada Ilaria[1], Turola Elena[2], Amin Ladan[3], Gabrielli Martina[2], D''Arrigo Giulia[1], Legname Giuseppe[3], Furlan Roberto[4], Cojoc Dan[5], Peruzzi Francesca[6], Verderio Claudia[1]

[1]CNR-IN Neuroscience Institute, Department of Medicine ~ Milan ~ Italy, [2]Department of Biotechnology and Translational Medicine, University of Milano ~ Milan ~ Italy, [3]Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA) ~ Trieste ~ Italy, [4]Institute of Experimental Neurology – INSpe, Division of Neuroscience, San Raffaele Scientific Institute ~ Milan ~ Italy, [5]CNR-IOM Institute of materials, Optical Manipulation Lab, , Area Science Park-Basovizza ~ Trieste ~ Italy, [6]Department of Medicine, Scott Cancer Center ~ New Orleans LA ~ United States

Beyond the classical secretory mechanism through which glial cells influence brain activity, astrocytes and microglia, release circular membrane fragments, known as extracellular vesicles (EVs). EVs contain several components of the donor cell (RNAs, proteins, lipids) and can transfer their cargo to recipient cells, functioning as an efficient intercellular delivery mechanism.

Aim of this study was to investigate whether glial cells may regulate neuronal gene expressions through EV secretion.

Primary cell culture, EV isolation by differential centrifugation, real-time PCR, Renilla/Luciferase-based assay, cell transfection, immunocytochemistry, western blot, optical manipulation and live imaging.

We identified a set of miRNAs differentially expressed in EVs produced by pro-inflammatory compared to pro-regenerative microglia. Among the glia-enriched microRNA we found, there is the miR-146a, which is altered in brain disorders and targets neuron-specific genes. To investigate a possible glia-to-neuron shuttling of miR-146a, we transfected hippocampal neurons with a miR-146a specific sensor for Renilla/Luciferase assay, and exposed them to glial-EVs for 24-72h. Exposure to glial EVs caused an increase in neuronal miR-146a levels, with a consequent decrease in the immunoreactivity of a validated miR-146a target, the synaptic vesicle protein synaptotagmin I. Transfection of donor glial cells with an anti-miR-146a inhibitor or clocking phosphatidyl serine residues, a determinant for EV recognition on neurons, on glial EVs themselves, resulted in unchanged mir-146a concentration in target neurons. Taken together, these data show that glia-derived EVs transfer biologically active mir-146a to neurons, highlighting the capability of glial cells to modulate neuronal gene expression. To investigate how the transfer of miRNA cargo takes place, we combined optical manipulation with live imaging. We observed that EVs positioned on the cell body make a quite stable interaction with neurons, remaining attached to the neuronal surface up to 1h. Together with confocal analysis of fixed neurons exposed to EVs for different time points, this observation rules out the possibility that EVs undergo rapid internalization or full fusion with cell membrane. Further investigation is ongoing to clarify whether EVs can open a transient pore to transfer their cargo to neurons.

Our data indicate that reactive astrocytes may influence neuronal activity by regulating the translation of a crucial component of the exocytotic machinery through secretion of miR146a-storing EMVs.

Symposium 21

ROLE FOR PPAR TRANSCRIPTION FACTORS IN THE ENERGETIC METABOLIC SWITCH OCCURRING DURING NEUROGENESIS

Di Giacomo Erica, Benedetti Elisabetta, Cristiano Loredana, D'Angelo Michele, Cerù Maria Paola, Cimini Annamaria

Derpt of Life, Health and Environmental Sciences, University of L'Aquila ~ L'Aquila ~ Italy

Neurogenesis takes place throughout life in two main areas of the adult mammalian brain: the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal formation. Although different, these two areas share an organized and specialized microenvironment, where Neural Stem Cells can interact with their progeny, blood vessels, ependymal cells, cerebrospinal fluid and other surrounding cells. This fine architecture defines the so-called “neurogenic niche” that provides signals regulating proliferation, migration and differentiation of NSCs and their progeny. Among these signals Peroxisome Proliferator Activated Receptors (PPARs) have been proposed to play an essential role in regulating NSCs proliferation and differentiation.

Since PPARs are known for being mainly involved in the regulation of energetic metabolism, the aim of this work was to correlate the modulation of the different PPAR isotypes with the energetic metabolic switch occurring during neurogenesis both in vivo and in vitro.

The experiments were conducted in vitro on human neuroblastoma cells, known to differentiate in neuronal-like phenotype under differentiation agents. Cells induced to differentiate were followed, for PPARs modulation, for different time points. On the chosen differentiation times, cells were investigated for glycogen and lipid content and for glucose utilization/storage enzymes by western blotting and immunofluorescence.
Cell were also silenced for PPARgamma and the same parameters were re-assayed.
The experiments were conducted also in vivo on 3 month old rat brain, were the localization of PPARs, glycogen, lipid droplets and key energetic enzymes was studied in the neurogenic niches.

The data obtained seem to indicate an involvement of PPARγ in the storage of glycogen and lipids in undifferentiated cells and that its inactivation is paralleled by the utilization of the energetic storages during the neuronal differentiation. In fact, during neurogenesis, both in vivo and in vitro, PPARγ inactivation parallels glycogen and lipid droplets consumption, while PPARβ/δ is increased and probably involved in the neuronal maturation, as previously demonstrated; PPARα is also increased, suggesting a function in the acquisition of the cholinergic phenotype. The data in vivo show the presence of PPARgamma in the neurogenic niche, particularly in stem cells. The immuno-positivity for the PPARgamma decreased in neuronal differentiated cells.

The data obtained point towards the involvement of these transcription factors in adult neurogenesis, thus adding new insight into the possible mechanisms underlying this crucial event, and possibly, suggesting that the modulation of these transcription factors by specific agonists/antagonists may be useful to induce neurogenesis in brain damaged areas.

COUP-TFI FUNCTIONS IN ADULT NEUROGENESIS

Bonzano Sara[1], Studer Michèle[2], De Marchis Silvia[1]

[1]Department of Life Sciences and System Biology and Neuroscience Institute Cavalieri Ottolenghi (NICO) ~ TORINO ~ Italy, [2]Institut de Biologie Valrose, Inserm U1091, CNRS UMR7277 and Univ. de Nice Sophia Antipolis (UNS), Faculté des sciences, Parc Valrose ~ Nice ~ France

The chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is a nuclear orphan receptor, highly expressed in embryonic neural tissues. Several studies have addressed its role during CNS development in mice, demonstrating distinct functions in neurogenesis, including control of neural stem/progenitor competence, neuronal differentiation, migration, axonal projection and cortical arealization. COUP-TFI continues to be expressed in adulthood as well, however its role in the adult age remains largely unknown.

The main goal of our project is to explore COUP-TFI functions in highly plastic brain regions in which neurogenesis occurs throughout life, namely the subventricular zone (SVZ)-olfactory bulb (OB) system and the hippocampal dentate gyrus (DG).

The cells expressing COUP-TFI in the SVZ-OB and DG of adult mice were characterized by quantitative co-localization analysis of confocal fluorescence microscopy images. To dissect COUP-TFI functions in these systems we took advantage of the Cre-loxP technology: floxed COUP-TFI mice were crossed with the inducible Glast::CreERT2 mouse line together with a reporter Rosa26-YFP line and the effect of COUP-TFI deletion analysed at 2 and 7 weeks post tamoxifen administration.

Analysis of COUP-TFI expression pattern revealed distinct features in the two neurogenic niches: COUP-TFI is widely expressed throughout the rostro-caudal axis of the DG, where it localizes in a large percentage (70-80%) of adult neural stem/progenitor cells and in virtually all neuroblasts and neurons in the granule cell layer. In contrast, its expression in the SVZ-OB system is restricted to a subset of progenitors -mainly cycling neuroblasts- confined to the SVZ dorsolateral domain, and to a subpopulation of adult born mature interneurons in the OB. Only rare o no COUP-TFI expression is found in migrating neuroblasts along the migratory stream toward the OB. These results suggest that COUP-TFI could play important and possibly diverse roles in the two neurogenic niches. The effect of selective deletion of COUP-TFI in adult neural stem/progenitor cells has been investigated in the DG, where we found an expansion of the radial glial/progenitor population paralleled by decreased neurogenesis, strongly supporting a major role for COUP-TFI in the control of adult hippocampal neurogenesis.

Ongoing experiments on the fate choice of neural stem/progenitor cells and their proliferation dynamics in absence of COUP-TFI are in progress to further elucidate COUP-TFI functions in adult neurogenesis.

Funding: Univ. of Turin [ex 60% 2013-2014]; Mobility grants: Campus France, Galileo project.

ALTERED SEROTONIN HOMEOSTASIS AFFECTS DEVELOPMENT AND MAINTENANCE OF SEROTONERGIC NEURONAL CIRCUITRY

Pasqualetti Massimo

Department of Biology, Unit of Cell and Developmental Biology, University of Pisa ~ Pisa ~ Italy

Serotonin is a neurotransmitter synthesized in two steps with tryptophan hydroxylase 2 (Tph2) as the rate-limiting enzyme and it is implicated in the modulation of numerous physiological processes including mood, sleep, aggressivity and sexual behavior. Serotonergic neurons provide a profuse innervation to the whole CNS. The synthesis of serotonin and the expression of its receptors early in embryonic development, as well as its maternal and placental source to the foetus, has led to the hypothesis that serotonin could act as a growth regulator in specific developmental events such as neurogenesis, neuronal migration and circuitry formation. However, the precise role of serotonin in specific morphogenetic activities on CNS development is only beginning to be elucidated.

The present work aims to investigate whether constitutive or inducible time-specific depletion of brain serotonin affects development of the CNS.

We have used a Tph2-GFP knockin mouse line in combination with a Tph2 conditional (floxed) allele to address the consequences of constitutive and time-controlled serotonin depletion on CNS development.

Results demonstrated that lack of serotonin produces in both newborn and adult mice severe abnormalities in the serotonergic circuitry formation within the rostral brain with a region and time-specific effect.

We have shown that the serotonergic system exhibits a previously unexpected plasticity and that appropriate serotonin homeostasis is crucial not only for proper development of the serotonergic neuronal circuit but also for its maintenance during adulthood.

ASCIDIANS AS A SIMPLE MODEL FOR THE STUDY OF DEVELOPMENT AND EVOLUTION OF VERTEBRATE NERVOUS SYSTEM

Pennati Roberta[1], Mercurio Silvia[1], Cauteruccio Silvia[2], Manenti Raoul[1], Licandro Emanuela[2], De Bernardi Fiorenza[1]

[1]Department of Biosciences, University of Milano ~ Milano ~ Italy, [2]Department of Chemistry, University of Milano ~ Milano ~ Italy

Ascidians (Tunicates) are marine animals whose common ancestry with vertebrates was recently recognized and is reflected in the tadpole-like larvae of most species. The advantages offered by their key phylogenetic position are enhanced by their anatomically simple and transparent embryos, compact genomes, and the availability of powerful experimental and computational tools with which to study these organisms. Moreover, developmental pathways for structures common in vertebrates and ascidians, like the nervous system, often involve the same molecules, even used in different ways. The larval nervous system is organized in a similar way as in vertebrates but is composed of less than 130 neurons and around 230 glial cells. This remarkable simplicity offers an opportunity to understand, at the cellular and molecular levels, the ontogeny and function of each neural cell.

We review the organization of the ascidian nervous system in comparison to that of amphioxus and vertebrates. Then we focus on the understanding of the processes of specification and patterning of the peripheral nervous system, mainly composed of multiple series of epidermal sensory neurons. In Ciona intestinalis, the most studied ascidian, we experimentally validated the spatio-temporal expression of some miRNAs, expressed preferentially in the nervous system of vertebrates.

We report the first results of knock down of miRNA-9, abundant in the mammalian brain and candidate to a regulatory role in nervous system development and differentiation, using a peptide nucleic acid as oligonucleotide mimic, directly injected in ascidian eggs to target miR-9.

PNA was able to affect the development of the peripheral nervous system by interfering with the activity of the neurogenic complexes of C. intestinalis larvae.

These results give insights into the evolutionary history of miRNAs in relation with the emergence of chordate nervous system novelties.

ORCHESTRATING CELL FATE DECISIONS IN PROGENITOR CELLS OF THE EMBRYONIC RETINA

Poggi Lucia

University of Heidelberg ~ Heidelberg ~ Germany

Reprogramming of retinal progenitor cells (RPCs) in vivo constitutes an attractive means for replacing lost retinal cells in humans. This needs that we understand how fate choices are orchestrated along the lineage history of an RPC during retinal development. With this aim, we combine time-lapse imaging in the retina of the developing zebrafish embryo with functional approaches to investigate cell-intrinsic and extrinsic events that restrict retinal ganglion cell (RGC) genesis in vivo.

Reprogramming of retinal progenitor cells (RPCs) in vivo constitutes an attractive means for replacing lost retinal cells in humans. This needs that we understand how fate choices are orchestrated along the lineage history of an RPC during retinal development.

With this aim, we combine time-lapse imaging in the retina of the developing zebrafish embryo with functional approaches to investigate cell-intrinsic and extrinsic events that restrict retinal ganglion cell (RGC) genesis in vivo.

We found staggering reproducible asymmetrical cell divisions linking RGC genesis to the genesis of other particular retinal subtypes. To begin to understand the molecular and cellular interactions restricting RGC fate acquisition by one daughter cell we identified cell division machinery components as intersecting point for intrinsic and extrinsic cues. We found a reciprocal feedback regulation of Anillin – essential F-actin binding protein and midbody component – and the transcription factor Ath5 – required for the specification of RGCs. Our study reveals that Anillin is required for proper apical cell domain inheritance and progenitor cell self-renewal: that is, anillin knock down favors symmetric neurogenic over asymmetric self-renewing cell divisions. Consequently, the retinal cell type composition is profoundly affected such that the number of RGCs is dramatically increased.

This study provides first in vivo evidence for a correlation between asymmetric inheritance of midbody components and cell fate in the retina. It also brings surprising insights into feedback loops of cytokinesis regulators and cell fate determinants, suggesting a mechanism whereby the competing needs of proliferation and differentiation are fine-tuned in the retina.

Symposium 22

ROLE OF NICOTINIC AND DOPAMINE D3 RECEPTOR CROSSTALK IN THE REGULATION OF DOPAMINERGIC NEURONS PLASTICITY

Bontempi Leonardo[1], Savoia Paola[1], Bono Federica[1], Fiorentini Chiara[1], Gotti Cecilia[2], Missale Maria Cristina[1]

[1]Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia ~ Brescia ~ Italy, [2]CNR, Institute of Neuroscience, Cellular and Molecular Pharmacology, Department of Medical Pharmacology, University of Milan ~ Milan ~ Italy

Dopaminergic neuron function is regulated by different receptors, including the dopamine D2-like receptors, such as the D3 receptor (D3R) and the acetylcholine nicotinic receptors (nAChR). In these neurons, evidence of a crosstalk between D3R and nAchR has been provided. It has been shown that D2R-like agonists may slow the progression of Parkinson's disease and that nicotine has neuroprotective effects on dopaminergic neurons. We have reported that nicotine regulates dopaminergic neuron plasticity by a mechanism involving the D3R. Our preliminary data demonstrated that in the substantia nigra the D3R co-immunoprecipitates with the Alpha4 subunit of nAChR suggesting that the functional interplay between the two receptors may be explained by the formation of a heteromeric complex.

The aim of this study was to investigate the molecular aspects of the D3R-nAChR interaction and the role of this complex in the modulation of the structural plasticity of dopaminergic neurons. Primary mesencephalic cultures from E12.5 mouse embryos, human embryonic kidney (HEK) 293 cells cultures, Bioluminescence Resonance Energy Transfer (BRET) and Proximily Ligation Assay (PLA) were used.

BRET experiments carried out in HEK293T cells expressing GFP2-tagged D3R and Rluc-tagged Beta2 or Alpha4 subunits showed that D3R specifically interacts with the Beta2 subunit of nAchR, indicating the formation of a heteromeric complex. The D3R/nAchR complex was visualized in primary mesencephalic neurons and in mouse substantia nigra sections by using PLA. Different interfering peptides were designed in order to indentify the specific receptor domains involved in this interaction. Two interfering peptides with amino acid sequences corresponding to the third intracellular loop of D3R (TAT-D3R) and the second intracellular loop of Beta2 nAChR subunit (TAT-Beta2) were produced. Both peptides significantly reduce the BRET ratio of D3R-GFP2/Beta2-Rluc in a dose dependent manner. PLA signal was also lost in mesencephalic neurons treated with both peptides. We also found that the neurotrophic effect of nicotine on dopaminergic neuron was specifically lost in the presence of the interfering peptides, indicating the involvement of the D3R/nAChR complex in supporting dopaminergic neuron plasticity.

Taken together these data demonstrate for the first time that in dopaminergic neurons, a direct interaction between D3R and Beta2 subunit of nAChR occurs to form a heteromeric complex that plays a crucial role in the control of dopaminergic neuron plasticity. This complex may thus represent the molecular target by which nicotine exerts its neuroprotective effects on dopaminergic neurons.

IDENTIFICATION AND CHARACTERIZATION OF NEW REGULATORS OF THE DOPAMINE SYSTEM IN THE ANIMAL MODEL C. ELEGANS

Lanzo Ambra, Di Schiavi Elia

IBBR, CNR ~ Napoli ~ Italy

Dopaminergic neurons are known to be crucial for brain functions. Dysfunction of the Dopaminergic system has been associated with a number of psychiatric and neurological diseases. Moreover the DA system plays a pivotal role in drug addiction. The molecular mechanisms underlying these diseases and dysfunctions are not completely understood. A deeper genetic analysis of DA system may be crucial to dissect the different functions played by DA neurons and to develop novel therapeutic approaches. To this end, researchers have to strive for a higher level of innovation using new animal models amenable for rapid genetic manipulations. We use the impressive experimental advantages offered by the animal model C. elegans, to understand how DA system develop, function, react and survive to various insults, in vivo, in the whole organism.

We aim at characterizing the physiological role played in DAergic neurons by candidate genes, using a reverse-genetic approach (Esposito et al., Gene, 2007) which allows to obtain an efficient neuron-specific knock-down.

We obtained by PCR-fusion and subsequent microinjection, transgenic lines in which candidate genes are silenced only in DAergic neurons. These lines were then tested for DA-mediated behavioral assay, such as the swimming-induced paralysis and the basal-slowing response. Possible neurodegeneration was assessed by using transgenic strains expressing GFP only in DAergic neurons.

We silenced in the DAergic neurons the unc-64/Stx1a gene, which encodes a SNARE protein, and whose full depletion causes a lethal phenotype. Animals lacking unc-64/Stx1a only in DAergic neurons were viable but presented a defect in DA-uptake and an alteration in amphetamine-induced paralysis, similarly to what observed in the dopamine transporter mutants. We took a similar approach with unc-63/CHRNA, an alpha-subunit of nicotinic acetylcholine receptor, expressed in muscles and in unidentified neurons. unc-63/CHRNA depletion causes a strong uncoordination and a partial resistance to the lethal effects of the nicotinic agonist DMPP. We silenced unc-63/CHRNA specifically in DAergic neurons and after exposing these animals to DMPP, we obtained a partial resistance to the lethal effects, which was similar to that observed in unc-63 loss of function mutants, thus demonstrating that unc-63 unexpectedly mediates nicotine toxic effects in DA neurons.

We identified new genetic and chemical modifiers of DA function, by genetic manipulations, drug treatments and detailed phenotypic analysis in vivo. Our data strongly support a new role played by unc-64/Stx1a and unc-63/CHRNA genes in the regulation of the DA system.

PRONOUNCED DOPAMINERGIC DYSREGULATION IN DOPAMINE TRANSPORTER KNOCKOUT RATS

Leo Damiana, Sukhanov Ilia, Illiano Placido, Mus Liudmila, Sotdnikova Tatiana, Espinoza Stefano, Gainetdinov Raul R

Istituto Italiano di Tecnologia ~ Genova ~ Italy

Dopamine (DA) plays an important role in the control of many vital physiological functions, including motor control, locomotion, emotional behavior. A variety of neuropsychiatric such as schizophrenia, Parkinson's disease and attention deficit hyperactivity disorder are widely accepted to have a basis in a dysfunction of the dopaminergic systems. Concentrations of DA in the synaptic cleft have been suggested to be the primary determinant of the intensity of neuronal signaling.

The major function of Dopamine Transporter (DAT) is the control of dopamine dynamics by rapid uptake of neurotransmitter into presynaptic nerve terminals. Therefore, DAT is an important regulatory element of both the synaptic action of DA and the intracellular stores of DA.Here, we present a newly developed strain of rats (DAT-KO rats) in which the gene encoding the DAT has been disrupted by using Zinc Finger Nuclease technology.

We used a combination of behavioural, neurochemical and molecular biology techniques to dissecy the DAT KO phenotype.

DAT-KO rats develop normally but have lower weight in comparison to heterozygote (HET) and wild-type (WT) rats. Like in DAT-KO mice, spontaneous locomotor activity is highly elevated in them. Expression of various genes involved in dopaminergic neurotransmission in the basal ganglia was tested by RT-PCR. D2 dopamine receptor mRNA levels are decreased in DAT-KO rats but expression of tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis, is down-regulated. HPLC tissue content analysis showed that DAT- KO rats have markedly decreased total tissue DA (and 3-4 fold increased levels of DA metabolites – DOPAC and homovanillic acid. Like in DAT-KO mice. Fast Scan Cyclic Voltammetry (FSCV) analysis of DA dynamics in the dorsal striatum revealed that DA clearance in DAT-KO is much longer (over 60 seconds) when compared with WT, suggesting that the increase in the spontaneous locomotor activity is a direct consequence of the extended length of time that DA spends in the extracellular space. Moreover, bath applied cocaine (3 uM) had no effect on evoked basal DA efflux on DAT-KO rats but it showed the well know increase in DA level in control animals. Inhibition of MAOs by pargyline seemed able to slow down DA clearance in KO animals.

In summary, lack of DAT in rats results in disrupted clearance of released DA that affects both the extracellular and intraneuronal concentrations of DA. DAT-KO rats could provide a novel translational model for several human diseases involving aberrant DA function and/or mutations affecting the DAT or DAT-related regulatory mechanisms.

HDAC INHIBITION REDUCES DOPAMINE-DEPENDENT NEURODEGENERATION IN A MOUSE MODEL OF DAT-DEFICIENCY SYNDROME

Ronzitti Giuseppe[1], Stefano Espinoza[2], Placido Illiano[2], Liudmilla Mus[2], Cinzia Magagnotti[3], Andrea Contestabile[2], Evelina Chieregatti[2], Angela Bachi[3], Raul R Gainetdinov[4]

[1]Genethon ~ Evry ~ France, [2]Istituto Italiano di Tecnologia ~ Genova ~ Italy, [3]Fondazione San Raffaele del monte Tabor ~ Milano ~ Italy, [4]Institute of Translational Biomedicine ~ St Petersburg ~ Russian Federation

Dopamine (DA) is a neurotransmitter with important physiological functions in the brain and linked to different pathological states. A loss in dopaminergic innervations is a key feature of Parkinson's disease, while enhanced dopaminergic tone has been linked to schizophrenia and Huntington disease. Recently, it has been found that mutations leading to the loss of function of the DA transporter (DAT) cause a severe neurological disease called DAT deficiency syndrome (DTDS). The disease manifests initially as hyperkinetic movement disorder that evolves in hypokinetic parkinsonism-dystonia. DAT-KO mice present symptoms similar to DTDS patients thus it is reasonable to expect that the molecular mechanisms involved in dopamine-dependent postsynaptic neurodegeneration in this mouse line are similar to those involved in the human pathology.

The DAT-KO mouse is an ideal model to define the pathways involved in dopamine-mediated neurodegeneration and the aim is use it to develop novel curative strategies for DAT-deficiency syndrome, thus meeting a precise medical need.

We used DAT KO mouse to mimic hyperdopaminergic tone and the dopamine-deficient DAT-KO mice (DDD) as an acute model of absolute dopamine deficiency. Two dimensional differential in-gel electrophoresis (2D-DIGE) followed by functional analysis of the identified proteins revealed acetylation as one of the pathways affected by the dopaminergic tone in striatum. Pharmacological inhibition of HDACs of class I and II has been used to counteract the dopamine induced neurodegeneration.

We found an up-regulation of several subtypes of histone deacetylase (HDAC) in DAT-KO mice, increase that was abolished in DDD-mice thus suggesting a dynamic regulation of these enzymes by the dopamine extracellular levels. Elevated dopamine levels in DAT KO mice have been related to the increase of tau phosphorylation and neurodegeneration, thus we decided to define the possible role of HDACs in this process. Pharmacological inhibition of HDAC activity decreased the level of phosphorylated tau in DAT-KO and the neurodegeneration associated to levels comparable to that of WT mice.

Our data indicate that the inhibition of HDAC represents a potential therapeutic option for dopamine-induced neurodegeneration, and suggest an intriguing role for dopamine in the regulation of HDACs.

Symposium 23

REMYELINATION IN STROKE: NEW TARGETS AND NEW CHALLENGES TO IMPLEMENT REPAIR AND FUNCTIONAL RECOVERY

Abbracchio Maria Pia[1], Bonfanti Elisabetta[1], Gelosa Paolo[2], Fumagalli Marta[1], Tremoli Elena[2], Viganò Francesca[4], Dimou Leda[4], Cimino Mauro[3], Luigi Sironi[1]

[1]Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Italy ~ Milan ~ Italy, [2]Centro Cardiologico Monzino IRCCS, Milan, Italy ~ Milan ~ Italy, [3]Dept. of Biomolecular Sciences, University of Urbino. ~ Urbino ~ Italy, [4]Physiological Genomics, Institute for Physiology and Ludwig-Maximilians University ~ Munich ~ Germany

Demyelination (i.e., loss of integrity of the myelin envelope ensheating neuronal processes) leading to impaired nerve conduction and neurological deficits is not only typical of multiple sclerosis, but represents a common feature of several neurodegenerative diseases, including stroke. The identification of new pharmacological targets to foster remyelination thus represents a new approach to implement repair and foster recovery in stroke. In this respect, Oligodendrocyte Precursor Cells (OPCs, the myelin forming cells) are still present in the adult CNS, and could be recruited to repair damaged myelin and restore cell-to-cell communication in the ischemic brain.

Along the years, our work has been aimed at (i) characterizing the new P2Y-like purinergic receptor GPR17 expressed by OPCs, and (ii) validating GPR17 as a new target for remyelinatin therapies. Within the activities of the ERANET-NEURON project “RENEW IT” coordinated by Elena Tremoli and by MPA, we have utilized a wide range of complementary techniques to analyze the role of GPR17 in ischemic damage associated to Middle Cerebral Artery occlusion (MCAo) in the rodent. Recently, we have extended our analysis to the new inducible reporter GPR17-iCreERT2xCAG-GFP mouse line for fate mapping studies. In these mice, upon tamoxifen administration, cells expressing GPR17 and their progeny can be visualized in vivo by GFP fluorescence, allowing to follow their final destiny.

In OPCs, GPR17 reaches its maximal expression peak at the stage of O4-positive immature OLs. Afterwards, GPR17 has to be downregulated, to allow cells to complete maturation. Changes of GPR17 were analyzed by confocal analysis during the post-ischemic period in the GPR17-iCreERT2xCAG-GFP mice. In detail, animals were sacrificed at different times (72 h, 1, 2, 4, 6 and 8 weeks) after MCAo and immunohistochemistry was performed. Our data show that the absolute number of recombined cells (GFP+ cells/mm2) significantly increased in the ipsilateral ischemic side starting from 72 h after MCAo compared to the contralateral side. At later times, some GFP+ cells were found to also express myelin markers, indicating that they had indeed progressed to more mature myelinating phenotypes.

These data suggest that the GPR17-expressing OPCs are extremely reactive to ischemic damage and directly involved in tissue remodelling. They also show, for the first time, that OPCs that have expressed GPR17 in their earlier life can indeed maturate in vivo to myelin producing oligodendrocytes, thus validating GPR17 as a new target for remyelinating therapies in stroke.

TARGETING TRANSCRIPTIONAL AND TRANSDUCTIONAL MECHANISMS CONTROLLING NA+/CA2+ EXCHANGER (NCX) AS NEW STRATEGY IN STROKE INTERVENTION

Annunziato Lucio

Department Neuroscience, School of Medicine, "Federico II" University of Naples ~ Naples ~ Italy

Changes in the Na+-Ca2+ exchanger 1 (ncx1) gene expression, a ubiquitous plasma membrane protein regulating cellular calcium and sodium homeostasis in the brain, is important for the progression of stroke and for the reduction of the cerebral infarct damage elicited by ischemic preconditioning. Indeed, studies have shown that ncx1 ablation markedly increases infarct volume after stroke and partially reverts preconditioning-induced neuroprotection. The reduction in the NCX1 expression observed in the ischemic brain of rats subjected to transient middle cerebral artery occlusion (tMCAO), is regulated at transcriptional and transductional level. Indeed, RE1-Silencing Transcription factor (REST), which regulates global gene expression after stroke, determines NCX1 downregulation, whereas, NCX1 increase in ischemic preconditioning plus ischemia (PC_tMCAO) is determined by hypoxia-inducible factor 1 (HIF-1).

ncx1 brain promoter (ncx1-Br) sequence contains several consensus binding sites for Specific protein 1 (Sp1). Sp proteins comprise four isoforms, Sp1-Sp4, which can act as either activators or repressors of gene expression. The presence of several binding sites for Sp1 on the ncx1-Br region and the fact that Sp1 is involved in NGF-induced regulation of NCX1 prompted us to investigate the relationship between the members of the Sp family and their role in modulating NCX1 expression during ischemia and during the endogenous neuroprotective strategy known as preconditioning (PC).

Brain ischemia was induced by 100’ of transient occlusion of the Middle Cerebral Artery (tMCAo). For the preconditioning experimental group, 3 days before harmful ischemia, animals were subjected to a subliminal 30’ tMCA0.

Our results indicated that NCX1 is epigenetically downregulated in brain ischemia by the REST/Sp3 complex and upregulated in brain ischemic preconditioning by the HIF- 1/Sp1 complex. Indeed, intracerebroventricular injections of siREST and siSp3, alone or in combination, in tMCAO, and of siHIF-1 and siSp1, alone or in combination, in PC+tMCAO completely reverted the decreases and the increases, respectively, in NCX1 mRNA and protein levels. Interestingly, a crosstalk between Sp1, Sp3, and REST specifically regulates ncx1 expression.

Under brain ischemia and brain ischemic preconditioning, NCX1 expression is epigenetically modulated, respectively, by two functional protein complexes: REST/Sp3/ HDAC1/HDAC2 and HIF-1/Sp1/p300. In particular, whereas the former downregulates NCX1 expression during brain ischemia, the latter upregulates it during preconditioning. Notably, the development of drugs that epigenetically regulate NCX1 by preventing its downregulation in stroke might be a new pharmacological avenue to ameliorate neuronal damage during brain ischemia.

VERSATILITY OF THE COMPLEMENT SYSTEM IN STROKE

Orsini Franca[1], Fumagalli Stefano[2], De Blasio Daiana[2], Zangari Rosalia[1], Zanier Elisa R[1], De Simoni Maria Grazia[1]

[1]IRCCS-Istituto di Ricerche Farmacologiche Mario Negri ~ Milan ~ Italy, [2]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico ~ Milan ~ Italy

Clinical and experimental evidence indicate that the complement system, a powerful arm of the inflammatory response, is involved to a different extent and with distinctive functions over time in acute brain injury. Data obtained by in vitro and in vivo approaches and in stroke patients will be presented to document the role of complement components, focussing on the lectin pathway (LP), one of the activation pathways of the complement system, and on its initiator molecules mannose-binding lectin (MBL) and ficolins.

To explore the role of MBL that is deposited on the ischemic endothelium after ischemia and whose inhibition is highly protective in mice, we have investigated: 1) the direct toxicity of MBL exposure to brain endothelial cells; 2) the consequences of MBL deletion following ischemia in mice. To explore the involvement of LP in human acute brain injury we have assessed LP initiators in subarachnoid hemorrhage (SAH) patients and related them to pathology and outcome.

In vitro: endothelial cells were exposed to oxygen-glucose deprivation (OGD) and then to MBL or vehicle. Cell death and MBL deposition were measured. In vivo: C57Bl/6J wild type (wt) or MBL-/- mice underwent transient middle cerebral artery occlusion. Blood flow speed and vascular permeability were measured by two-photon microscopy. Pro- and anti-inflammatory markers were measured in plasma and brain tissue. Patients: plasmatic concentration of LP initiators (MBL, ficolin-1,-2,-3) and their activity were measured in SAH patients and controls. SAH severity, occurrence of vasospasm and/or cerebral ischemia, CT scan, 6-month outcome were recorded.

In vitro: MBL induced endothelial cell death following OGD compared to vehicle. Toxicity was accompanied by 2-fold increase in MBL deposition on endothelial cells. In vivo: better flow recovery and less extravasation were observed in MBL-/- mice compared to wt. The anti-inflammatory thrombomodulin and CD206 were increased in brain tissue, while the pro-inflammatory ICAM-1 was decreased. Patients: the actual plasma concentration of ficolin-3 was related to SAH severity, vasospasm, and cerebral ischemia. Ficolin-3 functional LP activity was decreased in patients with unfavourable outcome.

The LP of the complement system plays a key pathogenetic role in brain ischemia and may be targeted to control injury progression.

CARBONIC ANHYDRASE IX AND ITS INTERACTOME IN HYPOXIA

Zambrano Nicola

Dipartimento di Medicina Molecolare e Biotecnologie Mediche and CEINGE Biotecnologie Avanzate ~ Napoli ~ Italy

Carbonic anhydrase IX (CA IX) is involved in survival and pH regulation in hypoxic cells. It is classically reported as a membrane protein, however, we have recently described a complex sub cellular localization for this enzyme in human cells, as well as its redistribution to nuclei and nucleoli during hypoxia. Accordingly, the interactome of CA IX is represented by several components of the nuclear transport machinery. We also highlighted the binding of carbonic anhydrase IX to nucleolar chromatin, which is regulated by oxygen levels. The search for selective CA IX inhibitors is a very active field in research, since several scientific reports associate CA IX function to the adaptation of cancer cells to hypoxic stresses. However, little is known on CA IX expression and function in the nerve system. CA IX could actually support survival of neurons during differentiation, and following hypoxic insults. Our most recent results indeed confirm a role for CA IX and its complex with exportin-1, one of its major interactors, during nucleolar stress and in the attenuation of rRNA synthesis in neuronal cells exposed to hypoxia.

Having characterized CA IX and its interactome at the molecular level, we are currently exploring the functions of this network of interacting proteins in neuronal differentiation and in the cellular responses to hypoxia.

We explored, via fluorescence microscopy and molecular tools, the subcellular distribution of CA IX and its interactors, as well as their representation in nuclei and nucleoli, and on the corresponding chromatin sites.

Our data show that the presence of nuclear CA IX accompanies the neuronal differentiation of murine embryo stem cells and of retinoic-acid treated SHSY-5Y neuroblastoma cells. Nuclear CA IX is also evident in neurons, in vivo. Hypoxic cells also show a decreased representation of CA IX on the nucleolar chromatin, but increased levels of its complexes with Exportin-1. This molecular picture is complemented by the decreased transcription of the 45S rRNA precursor and to translation attenuation, which is commonly observed in hypoxic cells as an adaptive response to the altered metabolism.

Our data support the relevance of CA IX and its selected interactors in neuronal survival during differentiation, and after hypoxic stress. We are now switching to in vivo analysis, to support these findings and their relevance in models of ischemic stroke.

Research supported by Associazione Culturale DiSciMuS RFC

Symposium 24

HYPERPOLARIZATION-ACTIVATED CYCLIC NUCLEOTIDE GATED CHANNELS AS A PROMISING NEW TARGET FOR PAIN TREATMENT

Resta Francesco, Masi Alessio, Micheli Laura, Laurino Annunziatina, Sili Maria, Di Cesare Manneli Lorenzo, Ghelardini Carla, Mannaioni Guido

Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence ~ Florence ~ Italy

Hyperpolarization-activated Cyclic Nucleotide gated (HCN) channels have been recently proposed as the “pacemakers” of pain, playing an important role in both inflammatory and neuropathic pain. Several studies reported enhanced HCN channels expression and Ih density in peripheral pain pathway following neuronal damage. Increased Ih activity could lead to a shifted resting membrane potential towards more depolarized values, thus facilitating spontaneous and/or repetitive neuronal activity, in line with the increased responsiveness to painful stimuli. Direct block of HCN channels could causes neuronal or cardiovascular side effects. Isorform-selective HCN channel blockers could be useful but unfortunately nowadays are still unavailable. Thus, alternative modulation of HCN channels could be crucial in treatment for both neuropathic and inflammatory pain.

We evaluated HCN-mediated current changes in a model of Chemotherapy-Induced Peripheral Neuropathy (CIPN) and tested the analgesic effect of the HCN blocker Ivabradine in an in vivo model of CIPN. Furthermore, we verified the possible modulation of HCN2 by decreasing intracellular cAMP formation through activation of G-protein coupled receptor 35 (GPR35) in a model of inflammatory pain.

DRG neurons were prepared as previously described by Vellani and co-authors. Rat dorsal root ganglion (DRG) neurons were isolated from neonatal (P7-P10) or adult (280-300 gr) wistar rats. Whole-cell recordings were performed on cultured DRG neurons 24 and 48 hours after isolation. Chemotherapy-Induced Peripheral Neuropathy was induced by a daily i.p. injection of 2.4 mg/Kg of Oxaliplatin for 15 days in adult wistar rats.

HCN channels-mediated current density was increased in small and medium size DRG neurons obtained from oxaliplatin-treated rats. Moreover, in these cells membrane potential was shifted towards more depolarized values thus leading to an increased neuronal excitability and a reduced latency in generating action potential. Interestingly, ivabradine exerted analgesic effect against oxaliplatin-induced mechanical hyperalgesia, allodynia and thermal hyperalgesia in vivo. Finally, we were able to modulate HCN channels through activation of GPR35 receptor in vitro and in vivo inflammatory pain models.

The results suggest an important contribution of HCN channels in CIPN and highlighted the possibility of an alternative modulation of HCN channels through activation of GPR35.

CONTRIBUTION OF THE H-CURRENT TO AUTORHYTHMIC ACTIVITY IN DOPAMINERGIC NEURONES OF SUBSTANTIA NIGRA AND OLFACTORY BULB

Pignatelli Angela, Gambardella Cristina, Belluzzi Ottorino

Università di Ferrara. Dipartimento di Scienze della Vita e Biotecnologie ~ Ferrara ~ Italy

The h-current (Ih) is present in most dopaminergic (DA) neurons, but how this current affect the excitability profile of these cells, and in particular its contribution to the resting membrane potential and pacemaker activity, is still uncertain under some aspects.

Our objectives were to investigate and compare the functional properties of the h-current in two types of dopaminergic neurons with very different characteristics, namely large projection neurons of substantia nigra - pars compacta (SNc) and small interneurones of the olfactory bulb (OB).

The identification of DA neurons was made possible by using a transgenic mice expressing eGFP under the tyrosine hydroxylase promoter. The h-current was kinetically characterized at 37 °C, using perforated patch-clamp recordings in thin slices.

The midpoint of activation was -82.7 mV in the OB and -77.5 mV in the SNc; in both cases we measured a significant fraction of h-channels open at rest, entailing a substantial contribution of this current to the resting potential, and suggesting a relevant function in the control of the cell excitability. The blockage of Ih induce a strong inhibition of spontaneous firing both in OB and SNc neurons. However, we show that this effect is not due to a direct role of the current in the pacemaker process, but to the depolarizing effect of Ih at rest.
The application of neurotransmitters (DA, 5-HT and noradrenaline) physiologically released onto SNc and bulbar neurones, and known to act on metabotropic receptors coupled to the cAMP pathway, was examined. The neurotransmitters tested had different effects on the Ih amplitude in the two populations of DA neurones studied. Direct activation of D2 and 5-HT receptors results in Ih inhibition in midbrain, but not in bulbar DA cells, where no measurable variation of Ih amplitude was observed; on the contrary, NA showed a significant inhibitory effect in both cases.

These data suggest that in both the DA populations examined, Ih does not participate directly to the pacemaker activity, but finely tune the resting membrane potential, thereby influencing the excitability profile of these cells; the modulation of Ih by endogenously released neurotransmitters –mainly but not exclusively linked to the cAMP pathway- contribute significantly to the control of the h-current -and consequently of DA neurons excitability- in both directions, possibly affecting the processing of information taking place in the circuitry hosting these cells.

DYSFUNCTIONAL HCN ION CHANNELS IN IDIOPATHIC EPILEPSY

Di Francesco Jacopo Cosimo

Neurological Inst. C. Besta ~ Milano ~ Italy

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are expressed as four different isoforms (HCN1-4) in the central and peripheral nervous systems. HCN channels are activated by membrane hyperpolarization at voltages close to resting membrane potentials and carry the hyperpolarization-activated current, dubbed Ih in neurons.

HCN channels contribute in several ways to neuronal activity and are responsible for many important cellular functions, including cellular excitability, generation and modulation of rhythmic activity, dendritic integration, transmission of synaptic potentials and plasticity phenomena.

Because of their role, defective HCN channels are natural candidates in the search for potential causes of epilepsy in humans.

Several experimental and clinical data, including growing evidence that some forms of epilepsy are associated with HCN mutations, support the notion of an involvement of dysfunctional HCN channels in different experimental models of the disease. Moreover, some anti-epileptic drugs are known to modify the activity of the Ih current.

Further studies are needed to better understand the pathogenetic mechanisms linking epilepsy to the dysfunction of HCN activity, potentially proposing these channels as possible therapeutic targets for the treatment of the disease.

THE HYPERPOLARIZATION-ACTIVATED CURRENT AS A DETERMINANT OF SELECTIVE NIGROSTRIATAL DEGENERATION IN PARKINSON'S DISEASE

Masi Alessio[1], Narducci Roberto[2], Francesco Resta[2], Carbone Carmen[2], Provensi Gustavo[2], Costa Alessia[2], Mannaioni Guido[1]

[1]NEUROFARBA, AOUC ~ Firenze ~ Italy, [2]NEUROFARBA ~ Firenze ~ Italy

Parkinson's Disease (PD) is caused by massive, selective degeneration of dopaminergic (DAergic) neurons in the Substantia Nigra pars compacta (SNc). In contrast, DAergic neurons in the neighbouring Ventral Tegmental Area (VTA) are much less affected. The bases of this peculiar aspect of the disease are still unclear, as the two DAergic subgroups share all the neurochemical and biophysical properties deemed critical in PD pathogenesis. Increasing evidence suggests that a complex SNc-specific interplay of pathogenic determinants, rather than individual factors, underlies selective vulnerability. We recently demonstrated that MPP+, a neurotoxin able to cause selective nigrostriatal degeneration in rodents and primates, alters the electrophysiological properties of SNc DAergic neurons in vitro by inhibiting the Hyperpolarization-activated current (Ih).

Based on these premises, the goal of this work is to identify molecular and physiological determinants of differential vulnerability within DAergic neurons.

Whole-cell recordings were performed in acute midbrain slices from juvenile WH rats or TH-GFP mice. Simultaneous determination of changes in cytosolic calcium concentration was achieved by loading the recorded neuron with Fluo-4 or Oregon Green. Fluorescence was elicited with a blue LED and detected with a photomultiplier tube.
Inactivation of Ih in vivo was obtained by stereotaxic intranigral injection of ZD7288 or ivabradine in adult WH rats or TH-GFP mice.

In midbrain DAergic neurons from TH-GFP mice, pharmacological suppression of Ih increases the amplitude and duration of evoked Excitatory Post-Synaptic Potentials (EPSPs). Moreover, Ih suppression leads to temporal summation of multiple EPSPs, indicating reduced ability to resolve single excitatory inputs at somatic level. The extent of this response depends on postsynaptic Ih magnitude and is significantly greater in SNc compared to VTA DAergic neurons. In vivo, local inactivation of Ih with specific blockers causes a DAergic degeneration pattern reminiscent of MPTP-intoxication.

These results indicate that Ih regulates dendritic excitability differentially within midbrain DAergic neurons and suggest that differential impact of MPP+-mediated Ih suppression may underlie selective vulnerability. In this respect, we have obtained preliminary experimental evidence that intranigral injection of Ih blockers recapitulates the DAergic degeneration pattern of PD. Based on these results, we propose that Ih loss of function, possibly resulting from metabolic stress in early phases of PD, may act in concert with SNc-specific connectivity to promote selective vulnerability.

Symposium 25

STRATEGIES TO ENHANCE THE CELL RESPONSE TO PROTEOTOXICITY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Crippa Valeria[1], D''Agostino Vito Giuseppe[2], Adami Valentina[3], Loffredo Rosa[3], Rusmini Paola[4], Cristofani Riccardo[4], Cicardi Maria Elena[4], Carra Serena[5], Provenzani Alessandro[2], Poletti Angelo[4]

[1]Experimental Neurobiology Lab, C. Mondino National Neurological Institute ~ Pavia ~ Italy, [2]Genomic Screeening Lab, Centre for Integrative Biology, Università di Trento ~ Trento ~ Italy, [3]High Throughput Screening core facility, Centre for Integrative Biology, Università di Trento ~ trento ~ Italy, [4]Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative (CEND), Universita' degli Studi di Milano ~ Milano ~ Italy, [5]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Universita' degli Studi di Modena e Reggio Emilia ~ Modena ~ Italy

Several brain disorders, including Alzheimer's, Parkinson, Huntington, Spinal and Bulbar Muscolar Atrophy (SBMA) and Amyotrophic Lateral Sclerosis (ALS) are characterized by the formation of proteotoxic aggregates in affected cells. Aggregates derive from inefficient removal of disease-related proteins that are prone to misfold. In physhiological conditions, cells have developed a system that avoids misfolded proteins accumulation: the protein quality control (PQC) system, that comprises molecular chaperones (like Heat Shock Proteins, HSPs) and the two degradative pathways, ubiquitin-proteasome system (UPS) and autophago-lysosome pathway (APLP). In brain disorders, a reduced activity of both UPS and APLP have been demonstrated; moreover both pathways have been found impaired by the presence of disease-related misfolded proteins. Thus, enhancing/restoring the PQC system response could counteract the accumulation of proteotoxic aggregates, being protective for misfolding protein diseases.
Focused the attention on the possible role of HSPs, an important PQC component, the small heat shock protein B8 (HSPB8), was found able to counteract misfolded proteins accumulation in Alzheimer's, Huntington and SBMA cellular models. Moreover, we found that HSPB8 is highly expressed in surviving motoneurons of transgenic ALS mice. In ALS cellular models, we demonstrated that HSPB8 overexpression prevents the aggregation of two ALS-related mutant proteins by increasing their autophagic degradation. For this activity, HSPB8 needs to complex with the co-chaperones BAG3, Hsc70 and the E3-ubiquitin ligase CHIP. The complex allows substrates recognition and degradation via APLP. Thus, HSPB8 could be a therapeutic target for ALS and others brain disorders.

Aim of the study was the identification of drugs that induce HSPB8 expression in motor neurons.

To study the HSPB8 gene transcriptional control, we developed a reporter assay based on murine motoneuronal (NSC34) and human neuronal (SH-SY5Y) cells stably transfected with a construct containing the human HSPB8 promoter (promB8) driving firefly luciferase expression. In collaboration with the Centre for Integrative Biology (CIBIO) in Trento, we performed a high throughput screening using a commercial library of 2000 compounds (The SPECTRUM Collection, MicroSource, USA).

We identified 18 putative hits. Active compounds were tested in NSC34 and SHSY5Y cells for 1) toxicity (MTT assay), 2) IC50, and 3) their ability to enhance endogenous HSPB8 expression (Real-Time PCR). We found two compounds that significantly increased HSPB8 mRNA levels, but not the levels of its co-chaperone BAG3, in human SHSY-5Y, in a dose-dependent manner. Interestingly, both compounds reduced ALS-related protein aggregation.

The two identified compounds could help to unravel HSPB8 role in the cell response to proteotoxicity.

EPIGENETIC ANALYSIS REVEALS IMPAIRMENT OF BDNF-6 EXPRESSION AND DENDRITIC TRAFFICKING IN KNOCK-IN BDNF VAL66MET MICE

Mallei Alessandra[1], Baj Gabriele[2], Ieraci Alessandro[1], Corna Stefano[1], Paiano Federica[1], Musazzi Laura[1], Lee Francis[3], Tongiorgi Enrico[2], Popoli Maurizio[1]

[1]Laboratory of Neuropsychopharmacology and Functional Neurogenomics - Dipartimento di Scienze Farmacologiche e Biomolecolari and Center of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano ~ Milano ~ Italy, [2]Department of Life Sciences, BRAIN Centre for Neuroscience, University of Trieste ~ Trieste ~ Italy, [3]Department of Psychiatry, Weill Cornell Medical College of Cornell University ~ New York ~ United States

The human Val66Met polymorphism in brain-derived neurotrophic factor (BDNF), a key factor in neuroplasticity, synaptic function and cognition, has been implicated in pathophysiology of neuropsychiatric and neurodegenerative disorders. The BDNF Val66Met transgenic mouse is the only existing animal model that recapitulates the phenotypic hallmarks of the BDNF Val66Met human polymorphism. Indeed, both human and mice BDNFMet allele carriers show reduced hippocampal volume, cognitive deficits, increased anxiety-related behaviour and impaired extinction of fear conditioning. BDNF is encoded by multiple transcripts with distinct regulation and localization, but the impact of the Val66Met polymorphism on BDNF regulation remains unclear.

Aim of the study was to investigate the regulation of expression and trafficking of the different BDNF transcripts in mice carrying the BDNF Val66Met human polymorphism.

In hippocampus from wild-type (BDNFVal/Val) and knock-in (BDNFMet/Met) mice we measured expression levels, epigenetic changes at promoters, and dendritic trafficking of distinct BDNF transcripts, using quantitative PCR, chromatin immunoprecipitation (ChIP), and in situ hybridization.

BDNF-4 and BDNF-6 transcripts were reduced in BDNFMet/Met mice, compared with BDNFVal/Val mice. Chromatin immunoprecipitations for acetyl-histone H3, a marker of active gene transcription, and trimethyl-histone-H3-Lys27 (H3K27me3), a marker of gene repression, showed higher H3K27me3 binding to exons 5, 6 and 8 promoters, in BDNFMet/Met. The H3K27 methyltransferase EZH2 resulted to be involved in epigenetic regulation of BDNF expression, because in neuroblastoma cells BDNF expression was increased both by siRNA for EZH2 and incubation with DZNep, an inhibitor of EZH2. In situ hybridization for BDNF-2, BDNF-4 and BDNF-6 after pilocarpine treatment showed that BDNF-6 transcript was virtually absent from distal dendrites of CA1 and CA3 regions in BDNFMet/Met mice, while no changes were found for BDNF-2 and BDNF-4.

We speculate that impaired BDNF expression and dendritic targeting in BDNFMet/Met mice may contribute to reduced regulated secretion of BDNF at synapses, and be a specific correlate of pathology in individuals carrying the Met allele.

EXPERIENCE-DEPENDENT DNA METHYLATION REGULATES PLASTICITY IN THE DEVELOPING VISUAL CORTEX

Napoli Debora[2], Tognini Paola[3], Tola Jonida[5], Silingardi Davide[5], Floriana Della Ragione[4], D''Esposito Maurizio[4], Pizzorusso Tommaso[1]

[1]NEUROFARBA, University of Florence ~ Florence ~ Italy, [2]BioSNS, Scuola Normale Superiore di Pisa ~ Pisa ~ Italy, [3]University of Irvine ~ Irvine ~ United States, [4]IGB A.B.T.CNR ~ Napoli ~ Italy, [5]Istituto di Neuroscienze CNR, Pisa ~ Pisa ~ Italy

DNA methylation is an epigenetic modification that consists in the addition of a methyl (-CH3) group to the fifth carbon of cytosines. DNA methyltransferases (DNMT) catalyzes this reaction altering the transcription status of the genes. Although it has been considered static for long time, recently many works demonstrate that DNA methylation is dynamically regulated in postmitotic neurons by electrical activity,during learning and memory, circadian rhythm and drug addiction.

Our aim is to assess if ongoing sensory stimuli could modify the epigenetic status of neurons in mouse primary visual cortex to modulate the transcriptional program necessary for experience-dependent plasticity.

We employed ocular dominance plasticity (ODP) induced by monocular deprivation (MD) during critical period as model of brain plasticity. After three days of MD, mice were studied with electrophysiological recordings and molecular analyses. We used the methylated and hydroymethylated DNA Immunoprecipitation (MeDIP and hMeDIP) followed by Real time-PCR and bisulfite sequencing to assess the epigenetic status of two well-known plasticity genes. Next, DNMT activity was blocked by pharmacological inhibitor and gene expression changes were studied by real time and moreover, by RNA-sequencing.

MD increased DNMT expression and decreased the mRNA level of cofactors (GADD45a, GADD45b and GADD45g) involved in the demethylation pathway. In agreement with DNMT upregulation, we found an increase of DNA methylation on the promoter regions of two well-known plasticity genes: BDNF exon IV and mir132. Deprivation of light stimuli exerted opposite effects on hydroxylmethylation of these two promoters. According to the epigenetic status of their regulatory regions, we found a downregulation of BDNF and mir132 transcripts. Inhibition of DNMT activity by RG108 infusion blocked this downregulation and also the downregulation of other 45 genes, assessed by next generation sequencing. Finally, we electrophysiologically tested ODP in monocular deprived mice that were infused with RG108 and we found no changes in ocular dominance physiology.

Taken together these data suggest that visual stimuli can regulate the epigenetic status of DNA on specific regulatory regions modifying the transcriptional program necessary for the molecular processes underlying ODP. In conclusion, DNA methylation can be used as molecular mediator in the experience-dependent refinement of cortical circuits during development.

LSD1/KDM1A MODULATES STRESS-EVOKED TRANSCRIPTION OF IMMEDIATE EARLY GENES AND EMOTIONAL BEHAVIOR

Rusconi Francesco[1], Grillo Barbara[1], Ponzoni Luisa[1], Bassani Silvia[2], Toffolo Emanuela[1], Reggini Valentina[1], Mallei Alessandra[1], Braida Daniela[1], Passafaro Maria[2], Popoli Maurizio[1], Sala Mariaelvina[1], Battaglioli Elena[1]

[1]University of Milan ~ Milan ~ Italy, [2]IN-CNR ~ Milano ~ Italy

Molecular mechanisms of stress response are poorly understood. Yet epigenetic modulation of stress-evoked transcription is emerging as critical in translating stressful stimuli into proper anxiety-like behavior, instrumental to respond to threatening conditions. Lysine-specific demethylase 1 (LSD1/KDM1A) and its dominant-negative splicing isoform neuroLSD1 have been implicated in setting excitability of the hippocampus, a brain area fundamental in the modulation of anxiety states.

We aimed at identifying LSD1 as a psychosocial stress transducer investigating its ability to modulate plasticity-related genes transactivation in response to social defeat stress.

By combining behavioral, chromatin and transcriptional analysis, we characterized mutant mice lacking neuroLSD1 as a model of defective stress response.

Here we demonstrate that LSD1/neuroLSD1 ratio orchestrates an epigenetic mechanism underlying the acquisition of normal anxiety-related phenotype. We show that in mouse hippocampus LSD1 and neuroLSD1 interact with the transcription factor SRF, setting the basal chromatin state of the SRF-targeted immediate early genes egr1 and c-fos. Indeed, we found that in mice complete lack (neuroLSD1KO) or reduction (neuroLSD1HET) of neuroLSD1 expression resulted in low anxiety-like behavior. On a molecular point of view, neuroLSD1 mutant mice display reduced levels of positive histone marks at the egr1 and c-fos promoters dampening their stress-induced transcription. Remarkably, anxiety phenotype and chromatin state can be reversed by pharmacologically inhibiting LSD1/HDAC2/CoREST corepressor complex.

We indicate LSD1 as a novel molecular transducer of stress stimuli, as well as a stress-response modifier. Indeed, LSD1 expression itself is acutely increased by stress at both transcriptional and splicing level. Our data provide a rationale to investigate LSD1 as a pharmacological target in the treatment of mood and anxiety disorders.

Symposium 26

IMPORTANCE OF TDP-43 N-TERMINAL REGION IN AGGREGATION

Buratti Emanuele

ICGEB ~ Trieste ~ Italy

TDP-43 is a 414 residue protein belonging to the heterogeneous nuclear ribonucleoprotein (hnRNP) family. In 2006, TDP-43 was found to form aberrant polyubiquitinated, hyperphosphorylated cytosolic aggregates which have been causatively linked to almost all ALS cases and approximately 60% of all FTLD cases. The protein contains an N-terminal domain, two RRM RNA-binding domains and a long, intrinsically disordered C-terminal region. From a structural point of view, several lines of evidence point toward its C-terminus as a key domain able to mediate this process. In keeping with this view, we have recently described a novel cellular TDP-43 aggregation model (TDP-43 12XQ/N) based on 12 tandem repetitions of its 339-366 Q/N rich prion-like domain. Notwithstanding the importance of the C-terminal region, the N-terminal domain has also been shown by several recent studies to affect self-interaction properties and splicing functionality of TDP-43.

The aims of our work are to functionally understand the structural-functional relationships between these two TDP-43 domains that lead to loss-of-function effects following aggregation. Specifically, it is our aim to experimentally determine which N-terminus residues of TDP-43 are essential to induce the loss of function effect observed within neuronal cells.

Following the expression and purification of residues 1-77 of TDP-43 using a bacterial expression system we have just obtained its essentially complete 1H, 15N and 13C NMR assignments. The structure consists of an alpha helix and six well-defined beta strands.

In parallel, using stable cell lines expressing various TDP-43 12XQ/N constructs, we have shown that a minimal sequence constituted by these N- and C-terminal regions, but lacking both RRM domains, can induce aggregation of endogenous TDP-43. Most importantly, this aggregation leads total loss of function effects as seen by changes in the alternative splicing of endogenous genes controlled by TDP-43. At present, we are currently in the process of integrating together both structural and functional observations to determine which N-terminus residues are essential for the loss-of-function effects.

Our results represent useful tools to better understand the role of TDP-43 N- and C-terminal domains in pathological aggregation. In particular, our results may eventually represent a first step in the development of small synthetic molecules capable of specifically affecting this process.

ALPHA-SYNUCLEIN AMYLOID INTERACTION WITH PRION PROTEIN: A PUTATIVE OVERLAP OF TWO NEURODEGENERATIVE DISEASES

Legname Giuseppe

Scuola Internazionale Superiore di Studi Avanzati, Italy

While the function of the cellular prion protein (PrPC) is still under debate, there are several reports indicating PrPC as being able to interact with Aβ oligomers.

Here, we investigate whether this is also true for α-synuclein (α-syn), protein involved in a group of pathologies collectively known as synucleinopathies.

We formed recombinant mouse α-syn amyloids using the same methodology employed to obtain synthetic mammalian prions. A combination of AFM (atomic force microscopy) and biochemical techniques was used to characterize the morphology and biochemical properties (such as resistance to proteinase K) of α-syn amyloids. Afterwards, we explored the uptake of α-syn amyloids in neuroblastoma cell line: N2a cells which endogenously express PrPC (N2aWT), the overexpressing PrPC (N2aPrPFL), knocked out for PrPC (N2aKO), and scrapie infected N2a (ScN2a) cells.

Our results show that the uptake of α-syn amyloids is lower in N2aKO if compared to control cells. Confocal microscopy and co-localization with sub-compartmental markers revealed that the α-syn amyloids co-internalized with PrPC, accumulated and trafficked to lysosomes. Moreover, internalized α-syn amyloids were co-localizing with scrapie deposits in ScN2a cells. Further work was required to validate the importance of this interaction in disease progression in vivo. Thus, we performed stereotaxic injections of α-syn amyloids in substantia nigra pars compacta and striatum in FVB PrPWT and FVB PrPKO mice. Our findings suggest a role for PrPC in regulating of α-syn uptake, thus, evidencing a link between the two neurodegeneration associated proteins.

This study suggests an overlap between prion disease and synucleinopathies.

UNRAVELING THE MOLECULAR BASIS OF PHENOTYPIC HETEROGENEITY OF ALZHEIMER DISEASE: THE MODEL OF PRION STRAINS

Di Fede Giuseppe

IRCCS Foundation "Carlo Besta" Neurological Institute ~ Milan ~ Italy

A remarkable aspect of the genetic forms of Alzheimer's disease (AD) is the considerable phenotypic heterogeneity that is due to variations of the cognitive profile and the occurrence of a broad spectrum of neurological deficits. Sporadic AD is commonly considered much less heterogeneous, presenting with a characteristic progressive amnestic disorder in most instances. However, other presentations, including behavioral, language or visual variants resulting in atypical clinical phenotypes, and occurrence of neurological abnormalities, such as extrapyramidal signs and myoclonus, are not infrequent.

The aim of this study is to unravel the molecular basis of phenotypic heterogeneity of AD. This is a critical aspect that has important diagnostic and therapeutic implications.

We carried out neuropathological studies on a series of patients with genetic and sporadic AD selected for having distinct clinical phenotypes, and found striking differences in brain regional distribution, topology, relative abundance and morphology of amyloid β (Aβ) deposits. To unravel the molecular counterpart of these differences, we used an immunoproteomic assay enabling detection of the whole panel of Aβ using SELDI-TOF MS. In a second set of experiments, brain extracts from AD patients having different Aβ profiles were inoculated in transgenic mice expressing human amyloid precursor protein (APP), or used as a template for in vitro conversion assays.

Analysis of brain extracts and purified amyloid fractions from prototypic neuropathological AD subtypes showed remarkable differences in composition of Aβ species, resulting in specific fingerprints for each phenotype. Amyloidogenesis can be induced in mice by introduction of an exogenous seed (i.e. AD brain extracts). This process is governed by host and source of the Aβ-seed, reminiscent of prion stain propagation. On this ground we inoculated human APP overexpressing mice with brain homogenates from patients with different molecular subtypes of AD, and found that the phenotypic diversity of human pathology was partly maintained in the inoculated mice. Furthermore, different Aβ "strains" showed distinct physicochemical and propagation properties in vitro.

These data suggest that the phenotypic heterogeneity of AD is related, at least in part, to the physicochemical and biological properties of specific Aβ species, similarly to the PrPSc strains in prion diseases.

ROLE OF TUNNELING NANOTUBES (TNTS) IN INTERCELLULAR SPREADING OF PRIONS AND OTHER PROTEIN ASSEMBLIES INVOLVED IN NEURODEGENERATIVE DISEASES

Zurzolo Chiara

Pasteur Institute ~ Paris ~ France

Neurodegenerative diseases (NDs) such as Prion disease, Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) are part of a larger group of protein misfolding disorders (PMDs) characterized by the progressive accumulation and spreading of protein aggregates of different sizes - oligomers, protofibrils or fibrils-, which ultimately can assemble into extracellular amyloid deposits and/or intracellular inclusions. The best-characterized example of PMDs is prion diseases, which are caused by the conversion of the normal form of the prion protein (PrPC), to a misfolded form (PrPSc) through "template conformation changes". Like in Prion diseases, misfolded forms of alpha synuclein, tau, Abeta and Htt proteins associated with AD, PD and HD can be transmitted experimentally in cellular and in animal models where it can act as 'seeds' to recruit the endogenous protein into aggregates (seeding process). However, the mechanism of intercellular transfer is still obscure.

We study the mechanisms by which prion-like protein aggregates transfer between cells in order to charachterize the pathogenesis of neurodegenerative diseses and understand their mechanism of progression in the brain.

We have set up co-culture of model neuronal cell lines and primary neurons, and use both confocal microscopy (in live and fixed cells) and FACS analysis to charachterize the mechanism of amyloidogenic protein transfer.

We have recently described a novel mechanism of PrPSc transmission through Tunneling Nanotubes (TNTs). TNTs are actin-based fine protrusions connecting sparse cells in culture and represents a novel mechanism of cell-to-cell communication. We showed that TNT are mediating both exogenous and endogenous PrPSc transfer between infected and naïve mouse neuronal cells and between bone-marrow dendritic cells and primary neurons. Furthermore, mutant polyQ Htt aggregates appear to highjack TNTs as well as fibrillar and oligomeric ASYN assemblies. I will discuss our novel data on the mechanism of TNT formation and their role in the spreading of different alpha synuclein assemblies involved in Parkinson's disease.

We have shown that TNTs mediate the intercellular spreading of different amyloidogenic aggregates. I propose that TNTs contribute to the progression of the pathology of neurodegenerative diseases associated with the spreading in the brain of misfolded protein assemblies.

Symposium 27

ABUSED DRUGS REGULATE DOPAMINE DA RELEASE IN THE STRIATUM THROUGHOUT PRESYNAPTIC NICOTINIC RECEPTORS

Rizzo Francesca Romana[1], Federici Mauro[1], Mercuri Nicola Biagio[2]

[1]IRCCS Fondazione Santa Lucia ~ Rome ~ Italy, [2]University of Tor Vergata ~ Rome ~ Italy

Amperomeric recordings of synaptic DA release in the striatum have already indicated that besides the classical potentiation of DA release cocaine, as well as, methylphenidate depress the dopaminergic signal and this depends on drugs concentration and application time (Federici et al., J Biol Chem. 2014; 289(1): 264-74). Later, voltammetric evidences have also demonstrated that cocaine depresses DA release by blocking presynaptic nicotinic receptors (Acevedo-Rodriguez et al., Front Synaptic Neurosci. 2014 Sep 4;6:19).

Here we examine whether the DA blockers and abused drugs methylphenidate and ecstasy (MDMA) have similar or different actions from those of cocaine.

We are using in vitro amperometric recordings of synaptic- and drug- induced DA release in mice striatal and ventral mesencephalic slices. To accomplish this, carbon fiber electrodes (World Precision Instruments GmbH, Berlin, Germany) connected with a World Precision Instruments MicroC will be used.

We report that similarly to cocaine, also methylphenidate and ecstasy (MDMA) depress synaptic DA release by blocking nicotinic receptors. In fact, once a clear depression of DA release was obtained by superfusing the nicotinic antagonist mecamylamine, the residual component of synaptic neurotransmitter release was potentiated by either methylphenidate and MDMA.
In addition, by using cocaine insensitive mice we also demonstrate that MDMA is a DA uptake blocker acting on the same site of cocaine on striatal dopaminergic terminals.

More complex actions of abused drugs on striatal DA release are detected, and these are mediated by the involvement of nicotinic receptors on presynaptic dopaminergic terminals.

ROLE OF MUSCARINIC M1 RECEPTORS IN DYT1 DYSTONIA

Maltese Marta[1], Madeo Graziella[1], Sciamanna Giuseppe[1], Martella Giuseppina[1], Bonsi Paola[1], Pisani Antonio[2]

[1]University of Tor Vergata ~ Rome ~ Italy, [2]Fondazione Santa Lucia IRCCS ~ Rome ~ Italy

DYT1 dystonia is a severe form of inherited dystonia caused by a deletion in the gene encoding the protein torsinA. Currently the medical therapy for this disorder is still largely unsatisfactory. Muscarinic receptor antagonists represent one of the few available treatments. However, the specificity of these drugs and their mechanism of action are not entirely clear.

To characterize the effects of antimuscarinic agents on short- and long-term synaptic plasticity of striatal spiny neurons recorded from mice with the DYT1 dystonia mutation.

We performed a systematic analysis of the effects of anticholinergic drugs on short- and long-term plasticity recorded from striatal medium spiny neurons from DYT1 dystonia knock-in (Tor1a+/Δgag) mice heterozygous for ΔE-torsinA and their controls (Tor1a+/+ mice).

Rescue of bidirectional striatal plasticity was obtained with M1-preferring antagonists, such as pirenzepine and trihexyphenidyl, and by applying the novel selective M1 receptor antagonist VU0255035. Conversely, biperiden and ethopropazine failed to restore plasticity. M1 receptor antagonists also counteracted the M1-dependent potentiation of NMDA current in striatal neurons.

Selective M1 muscarinic receptor antagonism restores synaptic plasticity deficits in the striatum of DYT1 dystonia mutant mice, and provides a mechanistic basis for the development of novel therapies for this severe inherited disorder.

THE FUNCTIONAL CROSS-TALK BETWEEN NICOTINIC AND GLUTAMATERGIC RECEPTORS ON DOPAMINERGIC NERVE TERMINLS IN THE RAT NUCLEUS ACCUMBENS

Pittaluga Anna, Grilli Massimo, Olivero Guendalina, Padolecchia Cristina, Bonfiglio Tommaso, Marchi Mario

DiFar ~ Genoa ~ Italy

Colocalized nicotinic cholinergic receptors (nAChRs) and specific glutamatergic receptor subtypes located at the nerve endings level can be functionally coupled and modulate the release of different neurotransmitters.

In particular the talk will focus on the localization and the pharmacological characterization of some native cholinergic and glutamatergic receptor subtypes present on the dopaminergic and glutamatergic nerve endings in the rat nucleus accumbens.

These conclusions are supported by data obtained using synaptosomes in superfusion, supplemented and integrated with other results achieved using some molecular biology and immuno-cytochemical approaches.

Our findings demonstrate that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamatergic receptors are functionally coupled to co-localized nAChRs. In particular, the functional cross talk bridging nicotinic and glutamate receptors originates either a synergistic or an antagonistic interaction among them as suggested by the finding that the neurotransmitter release elicited by the activation of some AMPA and NMDA receptors can be negatively or positively modulated in response to a brief incubation with nicotine or with other nAChR agonists. This effect occurs fairly rapidly (in the course of few minutes) and requires the involvement of the trafficking of AMPA and NMDA receptors. Inasmuch, this event takes place also at very low concentrations of nicotine, comparable with those present in the blood of the smokers, and involves the activation of several nAChRs subtypes.

We propose that the dynamic control operated by the activation of a cholinergic nicotinic receptors on the glutamatergic system here described may represent an important presynaptic adaptation following the administration of nicotine. The understanding of the role of this new nicotinic-induced fine tuning of synaptic signals might open new and interesting perspectives in terms of explaining the mechanisms that underlie some of the effects of nicotine.

G PROTEIN-COUPLED RECEPTORS OLIGOMERS IN THE BASAL GANGLIA: FROM SINGLE-MOLECULE FLUORESCENCE MICROSCOPY TO THERAPEUTIC PERSPECTIVES

Scarselli Marco

University of Pisa ~ Pisa ~ Italy

Accumulating evidence indicates that signaling molecules such as G protein-coupled receptors (GPCRs) might be organized in oligomeric or multimeric superstructures. Specifically, it has been demonstrated by different groups that dopaminergic, muscarinic and many other receptors can exist as homo- and hetero-dimers or higher-order oligomers.

Despite the high interest related to this field, our understanding of the biological role of these receptor complexes is still controversial and the question "Do GPCRs function as monomers, dimers or oligomers ?" is still unanswered. We try to address this question.

On this subject, the introduction of super-resolution fluorescence microscopies has allowed visualizing receptors as single proteins in their biological environment, and to determine whether they exist as monomers, dimers and/or higher-order oligomers in vivo. In particular, the Photoactivated Localization Microscopy (PALM) visualizes single molecules in dense samples and Single-molecule Tracking (SMT) sees how GPCRs move and interact in living cells in the presence of different ligands. By using these microscopies, it has been analyzed the plasma membrane localization and distribution of many different GPCRs, such as adrenergic, dopaminergic and muscarinic receptors.

We presented data using the Photoactivated Localization Microscopy (PALM) to visualize single molecules in dense samples, and Single-molecule Tracking (SMT) to see how GPCRs move and interact in living cells in the presence of different ligands. PALM has demonstrated that GPCR oligomerization depends on the receptor subtype, cell-type, actin cytoskeleton and other proteins. Conversely, SMT has revealed the transient dynamics of dimer formation, where receptors display a monomer-dimer equilibrium characterized by fast association and dissociation. At steady state, depending on the subtype, about 30-50% of the receptors are part of homodimeric complexes

The application of these novel super-resolution techniques are unveiling the biological meaning of different receptor homo and hetero-oligomers expressed in the basal ganglia with relevant consequences for the discovery of new drugs for some related pathologies such as Parkinson's disease.

FREE ORAL COMMUNICATIONS

Free oral communications session 1

SHORT-TERM MONOCULAR DEPRIVATION ALTERS GABA IN THE ADULT HUMAN VISUAL CORTEX

Lunghi Claudia[1], Emir Uzay[2], Morrone Maria Concetta[1], Bridge Holly[2]

[1]Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa ~ Pisa ~ Italy, [2]FMRIB, University of Oxford ~ Oxford ~ United Kingdom

Neuroplasticity is a fundamental property of the nervous system that is maximal early in life, within the critical period. Intrinsic GABAergic inhibition is necessary to trigger ocular dominance plasticity and to modulate the onset/offset of the critical period. GABAergic inhibition also plays a crucial role in neuroplasticity of adult animals: the excitation/inhibition balance in the primary visual cortex (V1) seems to modulate the susceptibility of ocular dominance to deprivation. In adult humans, short-term monocular deprivation strongly modifies ocular balance, unexpectedly boosting the deprived eye, reflecting homeostatic plasticity. However, no direct evidence is available to support the involvement of intrinsic GABAergic inhibition in homeostatic plasticity induced by visual deprivation.

Investigating how short-term monocular deprivation alters GABAergic inhibition in the adult human visual cortex.

We measured GABA concentration from a 2 x 2 x 2 cm voxel within the visual cortex (centered on the calcarine sulcus) and from a control parietal voxel (centered on the posterior cingulate cortex) in 19 adult human observers, using ultra-high field (7T) magnetic resonance spectroscopy (MRS) before and after 150 minutes of MD. GABA concentrations were quantified with LCModel using the unsuppressed water signal as reference. We coupled the MRS measurements with psychophysical testing of BR between orthogonal red and blue gratings (SF 2cpd, contrast 50%, size 2°) viewed through anaglyph goggles.

We found that after monocular deprivation resting GABA concentration decreased in V1 but was unaltered in a control parietal area. Importantly, across participants, the decrease in GABA strongly correlated with the perceptual boost of the deprived eye measured by binocular rivalry. We also found that, after deprivation, the concentration of GABA measured during monocular stimulation correlated with the deprived eye predominance.

Taken together these results suggest that reduction in GABAergic inhibition triggers homeostatic plasticity in adult human V1 after a brief period of abnormal visual experience. These results are potentially useful for developing new therapeutic strategies that could exploit the intrinsic residual plasticity of the adult human visual cortex.

LIGHT-INDUCED NEURODEGENERATION, AN INNOVATIVE APPROACH TO MODEL PARKINSON'S DISEASE

Maggio Roberto[1], Romeo Stefania[1], Vitale Flora[1], Viaggi Cristina[2], Vaglini Francesca[2], Lozzi Luca[3], Scarnati Eugenio[1]

[1]Biotechnological and Applied Clinical Sciences Department ~ L'Aquila ~ Italy, [2]Department of Translational Research and New Technology in Medicine ~ Pisa ~ Italy, [3]Department of Physical and Chemical Sciences ~ L'Aquila ~ Italy

Light pollution is defined as excessive and inappropriate introduction of artificial light by humans. Growing awareness of adverse impacts of artificial light on human health has led to recognise light pollution as a significant global environmental issue.

This study explores the effect of continuous exposure to bright light on neuromelanin formation and dopamine neuron survival in the substantia nigra.

Twenty-one days after birth, Sprague–Dawley albino rats were divided into groups and raised under different conditions of light exposure. At the end of the irradiation period, rats were sacrificed and assayed for neuromelanin formation and number of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra. Furthermore, catecholamine content was assayed in striatum.

The rats exposed to bright light for 20 days or 90 days showed a relatively greater number of neuromelanin-positive neurons. Surprisingly, TH-positive neurons decreased progressively in the substantia nigra reaching a significant 29% reduction after 90 days of continuous bright light exposure. This decrease was paralleled by a diminution of dopamine and its metabolite in the striatum. Remarkably, in preliminary analysis that accounted for population density, the age and race adjusted Parkinson's disease prevalence significantly correlated with average satellite-observed sky light pollution.

Based on these original findings, our working hypothesis is that chronic artificial light exposure may be a key environmental factor implicated in the preferential degeneration of dopamine neurons in PD. Such exposure in animals will provide a novel PD model, useful to study gene/environment interactions and cell death mechanisms.

PS-1 PROTEIN AND FREE L-ARG ENHANCE THE PROP BITTER TASTE RESPONSIVENESS BY ACTING AS "SALIVARY CARRIERS"

Melis Melania[2], Arca Massimiliano[1], Aragoni Carla[1], Crnjar Roberto[2], Tomassini Barbarossa Iole[2]

[1]Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari ~ Monserrato, Cagliari ~ Italy, [2]Dipartimento di Scienze Biomediche, Università di Cagliari ~ Monserrato, Cagliari ~ Italy

Genetic variation in the ability to taste the bitterness of 6-n-propylthiouracil (PROP) is a complex trait that has been used as a marker of general taste sensitivity and to predict food preferences and eating behavior. Sensitive and non sensitive individuals are defined tasters and non-tasters, respectively. The term "super-tasters" is used to distinguish individuals who perceive PROP as extremely bitter from those (medium tasters) who perceive PROP as moderately bitter. PROP tasting is primarily due to variants of TAS2R38 receptors, but can be correlated with variations in chemical composition of saliva. Recently, we found that the PROP phenotype is associated to salivary levels of specific proteins (Ps-1 and II-2) belonging to the basic proline-rich protein family (bPRP).

Here, we evaluated the role of Ps-1 protein as well as of free L-Arg, an amino acid highly represented in the Ps-1 sequence, in modulating PROP bitter taste responsiveness. We also characterized the chemical interaction between free L-Arg and the PROP molecule.

Fifty-one subjects were genotyped for TAS2R38 and classified by their PROP taster status by rating taste perception intensity evoked by PROP and NaCl solutions. Quantitative determinations of salivary levels of Ps-1 protein or L-Arg were performed by HPLC-ESI-MS analysis. PROP bitterness intensity and latency were assessed before and after oral supplementation of L-Arg at increasing concentrations. The chemical interaction between free L-Arg and the PROP molecule was investigated by 1H-NMR spectroscopy and quantum-mechanical calculations.

Salivary levels of Ps-1 protein were higher in saliva of PROP super-tasters and medium tasters than in non-tasters, and the oral supplementation of Ps-1 protein in individuals lacking it in saliva enhanced their PROP bitter taste responsiveness, and this effect was specific to the non-taster group. On the other hand, salivary levels of L-Arg are higher in PROP super-tasters compared to medium tasters and non-tasters, and the supplementation of free L-Arg enhances PROP bitterness intensity as well as reduces bitterness latency in a dose-dependent manner, particularly in individuals with low salivary levels of both free L-Arg and Ps-1 protein. These effects were related to both PROP taster phenotypes and TAS2R38 genotypes. 1H-NMR results and quantum-mechanical calculations showed that the –NH2 terminal group of the L-ArgH+ side chain interacts with the carbonyl or thiocarbonyl groups of PROP by forming two hydrogen bonds with the resulting charged adduct.

The formation of this PROP•ArgH+ hydrogen-bonded adduct could enhance PROP bitter taste responsiveness by increasing the solubility of PROP in saliva and its availability to receptor sites. These data suggest that Ps-1 and L-Arg could act as "carriers" of the PROP molecule in saliva.

A POLYMERIC NEURAL-INTERFACE RESTORES LIGHT SENSITIVITY AND VISUAL ACUITY IN ADULT BLIND ROYAL COLLEGE OF SURGEONS RATS

Maya Vetencourt José Fernando[2], Ghezzi Diego[2], Antognazza Maria R[1], Desii Andrea[1], Pertile Grazia[4], Mete Maurizio[4], Feyen Paul[2], Di Marco Stefano[3], Di Paolo Mattia[3], Bisti Silvia[3], Lanzani Guglielmo[1], Benfenati Fabio[2]

[1]Center for NanoScience and Technology@PoliMi, Istituto Italiano di Tecnologia ~ Milan ~ Italy, [2]Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia ~ Genoa ~ Italy, [3]Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università dell'Aquila ~ Aquilla ~ Italy, [4]UO Oculistica, Ospedale S. Cuore-Don Calabria ~ Negrar ~ Italy

The progressive degeneration of photoreceptors due to single mutations in anyone of over 100 genes (Retinitis pigmentosa) is one of the major causes of adult blindness in humans. Unfortunately, no clinical treatment exists for the majority of genetic retinal diseases affecting photoreceptors. A novel research line in our laboratory has exploited the use of conjugated polymers to generate an organic photovoltaic retinal prosthesis. We found that organic materials, in particular photovoltaic semiconducting polymers, are suitable for the generation of a fully organic retinal prosthesis.

We initially tested the efficacy of photovoltaic polymers in stimulating blind retinas explanted from albino rats with light-induced degeneration of the photoreceptor layer. To this aim, acutely dissected retinas were placed on the organic polymer in sub-retinal configuration. Light stimulation of the degenerate retina was observed by monitoring multi-unit activity with an extracellular electrode positioned in the retinal ganglion cell (RGC) layer. Electrophysiological recordings showed that a light stimulus 16-fold lower than the safe limit for pulsed illumination elicited intense spiking activity in degenerate retinas placed on polymer-coated substrates to levels indistinguishable from those recorded in control retinas. This suggests that the neural-interface could mimic functional photoreceptors in activating the processing of the inner retina and may be able to rescue normal light sensitivity. Inspired by these findings, we started an in vivo study by implanting the retinal prosthesis in the eye of rats bearing photoreceptor degeneration due to mutation in the MERTK gene (Royal College of Surgeons rats), a widely recognized animal model of human Retinitis pigmentosa. Preliminary experiments indicate that: (i) the retina remains attached over the entire region of the sub-retinal implant without inflammation or fibrosis; (ii) pupil constriction in the implanted blind rat is similar to the response of the non-dystrophic animal and higher than that in non-implanted dystrophic animals; (iii) local field potentials evoked by either flash- or pattern-stimuli in the visual cortex of implanted dystrophic rats are significantly increased as compared to non-implanted dystrophic animals but indistinguishable from those in non-dystrophic controls. These data point towards the reinstatement of both light sensitivity and visual acuity in implanted dystrophic animals. The rescue of visual functions in adult dystrophic animals after the implant was confirmed at behavioural level using the dark-light box test.

Our results broaden the possibility of developing a new generation of fully organic prosthetic devices for sub-retinal implants.

REPEATED 6-HZ CORNEAL STIMULATION PROGRESSIVELY INCREASES FOSB/ΔFOSB LEVELS IN THE LATERAL AMYGDALA AND INDUCES SEIZURE GENERALIZATION TO THE HIPPOCAMPUS

Carmela Giordano[1], Vinet Jonathan[1], Curia Giulia[1], Biagini Giuseppe[2]

[1]Department of Biomedical, Metabolic and Neural Sciences, Laboratory of Experimental Epileptology, University of Modena and Reggio Emilia ~ Modena ~ Italy, [2]Department of Neurosciences, NOCSAE Hospital, Modena, Italy

Exposure to repetitive seizures is known to promote convulsions, which depend on specific patterns of network activity. The contribution of limbic regions to repetitive seizures is still poorly defined. Simplified animal models, in which predictable responses are observed after electrical stimulation, may help in defining the role of the different cerebral regions involved in ictogenesis.

Our goal was to evaluate the changes in seizure phenotype and neuronal network activation obtained in a modified version of the 6-Hz corneal stimulation model of psychomotor seizures.

Mice received a corneal stimulation for 3s with at 6-Hz frequency and current intensity of 32mA, (interstimulation interval: 72h; 4 sessions). Seizures were video-EEG recorded and scored. FosB/ΔFosB was used as a marker of neuronal activation. FosB/ΔFosB-immunopositive cells were identified using dual-labeled immunofluorescence and a Leica SP2 AOBS laser scanning confocal microscope.

Video-electroencephalography showed that evoked seizures were characterized by a motor component and a non-motor component. Seizures appeared always in frontal cortex, but only at the fourth stimulation they involved the occipital cortex and hippocampus. An increase in seizure severity, characterized by loss of posture due to tonic-clonic generalized convulsions, was noticed after the second session, but total seizure duration was unexpectedly reduced after the first session. This finding was not explained by a change in the duration of convulsive seizures, which remained constant during the various sessions. The decrease in seizure duration was instead related to a shortening of non-motor seizures. FosB/ΔFosB immunostaining decreased in the hippocampal formation and parahippocampal cortices after the first 6-Hz stimulation. Conversely, repeated corneal stimulations resulted in a significant increase of FosB/ΔFosB immunoreactivity in the lateral amygdala after the fourth session. No significant changes were found in the subiculum, piriform cortex and striatum. In addition, double immunofluorescence with calcium/calmodulin-dependent protein kinase II antibody identified FosB/ΔFosB-immunopositive cells mainly as principal neurons.

Our findings suggest a predominant role of amygdala in promoting progressively more severe convulsions as well as the late recruitment of the hippocampus in seizure spread. We propose that the repeated 6-Hz corneal stimulation model may be used to investigate some mechanisms of epileptogenesis and to test putative antiepileptogenic drugs.

Free oral communications session 2

DRUG DEVELOPMENT STRATEGY FOR NEURODEGENERATIVE DISEASES

Mandel Silvia

Teva Pharmaceutical Industries ~ Netanya ~ Israel

Converging evidence suggests that the pathophysiology of neurodegenerative diseases (NDDs) begin years, if not decades, prior to the onset of clinical symptoms, including memory impairment, motor disturbances and non-motor related abnormalities. Therefore, individuals at very early stages are the most likely to benefit from disease-modifying therapies should they become available. Thus far, no available treatments have been convincingly presented an ability to delay or restore neuronal degeneration. Currently, NDDs are viewed as multi-etiological disorders with a concomitant occurrence of several pathogenic mechanisms and thus, the main challenge is to find the meaningful biological targets for new diagnostic and drug development.

In my talk I will elaborate on notable developments in early and late-stage to address unmet needs in NDDs as Alzheimer's and Parkinson's diseases.

A survey of therapeutic strategies in the industry pipeline from neuroprotective/disease-modifying therapies to polypharmacy, rescue therapies and reformulation of existing drugs.

Significant efforts are put in the development of novel drugs engaging biological targets with a potential for neuroprotection and to address symptomatology with compounds directed towards biochemical systems that might contribute to a significant relief in patient's quality of life.

Disease-modifying/neuroprotective therapies would be a landmark development in the treatment of NDDs. Despite the promising findings with leading compounds in preclinical animal studies showing delay or restoration of neuronal degeneration and function, this has not translated into the clinical arena. Choosing the optimal drug targets remains a central challenge. Significant advances are also being made in developing symptomatic treatments for motor disability, cognitive deterioration, NDD-associated psychosis and autonomic dysfunction. Lastly, clinical studies selecting the subject population and implementation of biomarkers will notably impact the likelihood of success of new products.

RNA-THERAPEUTICS OF GENE HAPLOINSUFFICIENCIES

Fimiani Cristina[1], Goina Elisa[2], Mallamaci Antonello[1]

[1]SISSA ~ Trieste ~ Italy, [2]SISSA/ICGEB ~ Trieste ~ Italy

Gene haploinsufficiency is the key causative factor of a number of neurodevelopmental and neurological diseases. Transcriptional hyperstimulation of the spared allele could be a straightforward approach to fix these disorders. Nowadays, this might be achieved by three platforms, based on ZF-, TALE- and CRISPR-type artificial transactivators, as well as by siRNA/miRNA-like, small activating RNAs (saRNAs). However, artificial transactivators pose serious delivery issues due to their large size. Moreover, the trascriptional gain they elicit is hardly controllable. On the other side, the selection of effective saRNAs is a tricky task and molecular mechanisms underlying their function are still poorly known.

Two were our main aims: - developing a novel, RNA-programmable transactivator prototype, circumventing the main issues which prevent the exploitation of established platforms for therapy of haploinsufficiencies; - proving the saRNAs suitability to rescue gene haploinsufficiencies and reconstructing key molecular events which mediate their activity.

These aims were addressed in primary cultures of murine and human neural cells as well as in EBV-immortalized blood cells, engineered by lentiviral vectors and TetON technology. The outcome of manipulations was assessed by evaluating their primary molecular effects as well as by scoring histogenetic consequences of them. We developed a novel ribonucleoproteic transactivator prototype (NMHV),recognizing its target gene via a dedicated RNA cofactor. Thanks to this device, we specifically upregulated a number of genes of neurological interest. TheyincludeEmx2 and Foxg1, namely two key players mastering cortico-cerebral development. Gene upregulation was small, often around two-folds, and restricted to cells normally expressing the target gene. However, it robustly inhibited neuronal differentiation of pallial precursors, forcing them to proliferate. We exploited miRNA-like saRNAs to promote transcription of FOXG1 and FXN. Haploinsufficiency for these genes leads to two devastating neurological syndromes, the Rett syndrome and Friedreich ataxia (FRDA), respectively. As above, gene upregulation was small and restricted to cells expressing the target gene. Now, we are testing a selection of these saRNAs in vivo and we are addressing mechanisms underlying their action.

NMHV transactivators and saRNAs share two properties, (1) low and easily controllable transcriptional gain and (2) activity restricted to the expression domain of the gene of interest. These properties make them an appealing tool for "clean" functional rescue of haploinsufficiencies. To make these devices suitable for therapy, an in depth characterization of their functional properties and a proof-of-principle in vivo assessment of their power are needed.

A LONGITUDINAL DIFFUSION KURTOSIS IMAGING IN TRANSGENIC MOUSE MODEL OF PARKINSONS DISEASE OVEREXPRESSING ALPHA SYNUCLEIN

Khairnar Amit[1], Kucerova Jana[2], Drazanova Eva[3], Latta Peter[4], Szabó Nikoletta[5], Arab Anas[2], Hutter--Paier Birgit[6], Havas Daniel[6], Windisch Manfred[7], Sulcova Alexandra[2], Starcuk Jr Zenon[3], Rektorova Irena[1]

[1]Applied Neuroscience Research Group, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic ~ Brno ~ Czech Republic, [2]Experimental and Applied Neuropsychopharmacology Group, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic ~ Brno ~ Czech Republic, [3]Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno, Czech Republic ~ Brno ~ Czech Republic, [4]Multimodal and Functional Imaging Laboratory, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic ~ Brno ~ Czech Republic, [5]Department of Neurology, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary ~ Szeged ~ Hungary, [6]QPS Austria GmbH, Grambach, Austria ~ Graz ~ Austria, [7]NeuroScios GmbH, Graz, Austria ~ Graz ~ Austria

Treatment options for PD are limited to drugs elevating the dopamine levels in the brain by various mechanisms. Therefore, development of innovative neuroprotective treatments is a central challenge for future PD therapy which may halt or reverse the progression of disease. To evaluate efficacy and to monitor disease-modifying effects, reliable surrogate markers for early PD diagnosis and progression are needed when patients might still be eligible for neuroprotective treatment. Diffusion kurtosis imaging (DKI) an advanced MRI method by measuring non- Gaussian diffusion of water allows non-invasive in vivo assessment of tissue microstructure.

The aim of the present study was to evaluate age dependent changes induced by α -synuclein accumulation in gray matter of transgenic mouse model of PD (TNWT-61) with the help of DKI. We hypothesized that DKI by measuring non-Gaussian diffusion may better characterize the microstructural changes induced by α-synuclein aggregates in the TNWT-61 model.

TNWT-61 mice and wild type (WT) littermates (3, 9 and 14 months old) underwent DKI scanning using 9.4 T Bruker system in vivo. Region of interest analysis was used to compare kurtosis, diffusivity and fractional anisotropy maps in substantia nigra, striatum, hippocampus, sensorimotor cortex and thalamus of TNWT-61 mice and WT mice. Immunohistochemistry for α -synuclein was performed in 5 TNWT-61 mice (14 month old mice) and correlated with DKI findings.

TNWT-61 at all ages showed a significant increase in kurtosis metrics as compared to WT littermates. Whereas we found the decrease in diffusivity metrics only at the late stage of pathology in TNWT-61 (14 month old) compared to WT mice. With immunohistochemistry analysis in 14 month old TNWT-61 mice we found strong expression of human α -synuclein in substantia nigra, striatum, hippocampus, sensorimotor cortex and thalamus. However we found significant correlations between α -synuclein accumulation and increase in kurtosis and decrease in diffusivity only in the thalamus.

Diffusion kurtosis parameters by assessing non-Gaussian diffusion can provide improved sensitivity and better characterization of microstructural changes that may complement the traditional diffusion tensor imaging (DTI) parameters. Taken together, DKI seems to be a reliable

imaging biomarker to accurately assess the α -synuclein pathology and it may potentially improve the early detection of PD and help in monitoring its progression by characterizing specific gray matter brain changes.

SILENCING OF UPREGULATED KV3.4 POTASSIUM CHANNELS REDUCES ASTROCYTE ACTIVATION, SS-AMYLOID LEVEL IN TG2576 MICE

Ciccone Roselia, Pannaccione Anna, Boscia Francesca, Casamassa Antonella, Franco Cristina, Vinciguerra Antonio, Di Renzo Gianfranco, Annunziato Lucio

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples ~ Naples ~ Italy

Astrocyte dysfunction emerges early in Alzheimer's disease (AD) and may contribute to its pathology and progression. It has been proposed that reducing elevated levels of glial fibrillary acidic protein (GFAP) in astrocytes may provide a promising strategy to control neuronal dysfunction during the early stages of AD. Recently, the voltage gated potassium channel KV3.4 subunit underlying the fast inactivating K+ currents (IA), has been recognized to be relevant for AD pathogenesis and is emerging as a new target candidate for AD.

In the present study we investigated the expression and functional activity of the KV3.4 subunit in astrocytes.

To this aim we evaluated: 1) KV3.4 and GFAP protein expression by means immunofluorescence and western blot analysis; 2) functional activity of KV3.4 subunit by means patch-clamp and 3) the possible interaction between KV3.4 and GFAP by means and co-immunoprecipitation studies.

The KV3.4 protein expression and the activity were significantly upregulated in primary astrocytes exposed to β-amyloid1-42 fragment (Aβ-42). Furthermore, KV3.4 expression was intensely up-regulated in the astrocytes of the hippocampus, corpus callosum, and cerebellum areas of six-month-old Tg2576 AD mice at the early stages of AD. Coexpression and co-immunoprecipitation studies revealed a significant overlap and direct binding between KV3.4 and GFAP. Conversely, the selective knockdown of KV3.4 expression significantly down-regulated both GFAP and Aβ protein levels in the brain of Tg2576 AD mice.

Our results demonstrated that KV3.4 could have a critical role in astrocyte activation during the early stages of Tg2576 AD mice. Therefore, modulating astrocyte activity through a regulation of KV3.4 subunit functioning might open new avenues for developing innovative therapies capable of actually slowing down the progression of AD at its earliest stages.

AMBROXOL-INDUCED RESCUE OF DEFECTIVE GLUCOCEREBROSIDASE IS ASSOCIATED WITH INCREASED LIMP-2 AND SAPOSIN C LEVELS IN GBA1 MUTANT PARKINSON'S DISEASE CELLS

Ambrosi Giulia, Ghezzi Cristina, Zangaglia Roberta, Levandis Giovanna, Pacchetti Claudio, Blandini Fabio

Center for Research in Neurodegenerative Diseases, C. Mondino National Neurological Institute ~ Pavia ~ Italy

Heterozygous mutations in GBA1 gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are a major risk factor for sporadic Parkinson's disease (PD). Defective GCase has been reported in fibroblasts of GBA1-mutant PD patients and pharmacological chaperone ambroxol has been shown to correct such defect.

The objective of this study was to analyze the impact of GBA1 mutations in the context of PD by investigating GCase and specific lysosomal factors supporting GCase activity in fibroblasts of PD patients with or without GBA1 heterozygous mutations or healthy controls. Moreover, we sought to obtain further information on the effects of ambroxol in this context, to confirm its potential as a compound targeting lysosomal dysfunctions that may be central to PD pathogenesis.

We assessed protein levels of GCase, lysosomal integral membrane protein-2 (LIMP-2), which mediates GCase trafficking to lysosomes, GCase endogenous activator saposin (Sap) C and parkin, which is involved in degradation of defective GCase. We also measured activities of GCase and cathepsin D, which cleaves Sap C from precursor prosaposin.

GCase activity was reduced in fibroblasts from GBA1-mutant patients and ambroxol corrected this defect. Ambroxol increased cathepsin D activity, GCase and Sap C protein levels in all groups, while LIMP-2 levels were increased only in GBA1-mutant PD fibroblasts. Parkin levels were slightly increased only in the PD group without GBA1 mutations and were not significantly modified by ambroxol.

Our study confirms that GCase activity is deficient in fibroblasts of GBA1-mutant PD patients and that ambroxol corrects this defect. The drug increased Sap C and LIMP-2 protein levels, without interfering with parkin. These results confirm that chemical chaperone ambroxol modulates lysosomal markers, further highlighting targets that may be exploited for innovative therapeutic strategies for PD.

Free oral communications session 3

DOI MODULATES STRIATAL PLASTICITY BY INDUCING ERK PHOSPHORYLATION IN STRIATUM THROUGH 5-HT2A/C RECEPTORS IN PREFRONTAL CORTEX

Espinoza Stefano[1], Ronzitti Giuseppe[1], Caffino Lucia[2], Chieregatti Evelina[1], Fumagalli Fabio[2], Raul Gainetdinov[1]

[1]Istituto Italiano di Tecnologia ~ Genova ~ Italy, [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano ~ Milano ~ Italy

The extracellular signal-regulated kinase (Erk) phosphorylation plays a fundamental role in the molecular mechanism underlying the striatal plasticity. In particular the blockade of the Erk pathway prevents the establishment of long-lasting mechanisms, such as those that are at the base of the psychomotor sensitization and drug addiction. Serotonin (5HT) and in particular 5HT2A/C receptor ligands can modulate the striatal functionality e.g. by increasing dopamine release. Moreover, the pharmacological modulation of the 5HT2A/C receptors has been proposed as a novel strategy for the relapse of drugs addiction.

The aim of our work was to evaluate the effect of the classical 5-HT2A/C agonist DOI in striatal functionality and plasticity.

We inject DOI i.p. or locally in prefrontal cortex or striatum in C57Bl/6J mice to evaluate the effect of Erk phosphorylation by western blot. We used the BAC transgenic mice expressing the EGFP or Tomato under the D2 or D1 dopamine receptor promoter to study in which type of striatal neurons DOI induces Erk phosphorylation. We performed amphetamine induced sensitization to study DOI/amphetamine interaction in vivo. By western blot we also evaluate the expression of different proteins of the glutamatergic synapse.

The administration of DOI induced an increase of pErk2 in the striatum at the intermediate doses (1 and 3 mg/kg) but not at the lowest and the highest doses (0.3 and 10 mg/kg). Pretreatment with the selective MEK inhibitor SL327 was able to slightly reduce head twitches but not the locomotor activation induced by DOI. By immunohistochemistry, we found that DOI induced pErk mainly in the dorsolateral striatum and in both D1 and D2 expressing neurons. Then, to understand which area was responsible for this effect, we locally injected DOI in prefrontal cortex or in striatum. We found that only the injection in the prefrontal cortex was able to increase pErk in striatum. To understand what could be the behavioral effect of the DOI-induction of pERK we pretreated the animals with DOI and then evaluate amphetamine-induced sensitization. We found that DOI was able to increase the sensitization process in a Erk-dependent way. Finally, we found that DOI administration was able to change the expression of some subunits of the glutamatergic synapse in the striatum.

These results show that activation of 5-HT2A/C receptors in the prefrontal cortex induces Erk2 phosphorylation in the striatum and modulates striatal plasticity.

DIURNAL OSCILLATION OF SYNAPTIC WIRING OF OREXINERGIC NEURONS IN MICE

Laperchia Claudia[1], Imperatore Roberta[2], Bertini Giuseppe[1], Grassi--Zucconi Gigliola[1], Cristino Luigia[2], Bentivoglio Marina[1]

[1]Dept. of Neurological and Movement Sciences, University of Verona ~ Verona ~ Italy, [2]Institute of Biomolecular Chemistry, Endocannabinoid Research Group, CNR ~ Naples ~ Italy

Neurons containing the hypocretin/orexin (OX) peptides, located in the lateral hypothalamus, are key regulators of several physiological functions, including arousal and energy homeostasis, which have been related to motivated behavior regulation. Several findings have indicated that the activity of orexinergic neurons increases during wakefulness, thus pointing to a critical role in promoting and sustaining wake. However, how the activity of orexinergic neurons is regulated at the presynaptic level in basal conditions in relation with the sleep-wake and rest-activity alternation during 24 h remains to be determined.

Our study was prompted by the working hypothesis that plastic changes of the wiring of orexinergic neurons could underlie diurnal changes of their activity. To test this hypothesis, we here analyzed quantitatively excitatory vs inhibitory presynaptic inputs to the somata of orexinergic neurons in adult mice in basal conditions, sacrificed during the day (corresponding to the rest-sleep time in nocturnal rodents) and the night (the activity-wake time).

Male C57BL/6J mice of 3-6 months of age were randomly divided in two groups maintained under a 12h/12h light/dark or dark/light cycle, respectively, and sacrificed during the day (Zeitgeber time, ZT 3-4; ZT 0 corresponds to the lights-on time) or the night (ZT 15-16). Cryostat-cut coronal, adjacent brain sections (10 micron-thick) were processed for triple immunofluorescence with the following primary antibodies: OX-A to label orexinergic neuronal cell bodies; VGAT to label GABAergic elements or VGlut to label glutamatergic elements; synaptophysin to label synaptic endings. With this strategy, inhibitory synapses were defined by the colocalization of VGAT and synaptophysin, and excitatory synapses by the colocalization of VGlut and synaptophysin. Density of either type of synapse per micron of the membrane of OX-A cell bodies was analyzed on cells (n= 60 per animal) sampled in 4 focal planes in fluorescence microscopy followed by image deconvolution.

The findings showed that the total number of synaptic terminals apposed to OX-A cell bodies did not vary between day and night. However, interestingly, we found a switch from a significant prevalence of inhibitory inputs at daytime to a significant prevalence of excitatory inputs at night.

The present findings point out a striking diurnal oscillation of the excitatory and inhibitory synaptic terminals apposed to the somata of orexinergic neurons, which correlates with the animal's state-dependent behavior. The data open interesting questions on the synaptic plasticity of orexinergic neurons and on the mechanisms underlying such phenomenon.

QUANTITATIVE ANALYSIS OF THE CO-DISTRIBUTION OF GLT-1 AND NA+,K+-ATPASE ALPHA ISOFORMS IN RAT CEREBRAL CORTEX

Melone Marcello[1], Ciriachi Chiara[1], Conti Fiorenzo[2]

[1]Department of Experimental and Clinical Medicine, Section of Neuroscience and Cell Biology, Università Politecnica delle Marche ~ Ancona ~ Italy, [2]Department of Experimental and Clinical Medicine, Section of Neuroscience and Cell Biology, Foundation for Molecular Medicine, Università Politecnica delle Marche ~ Ancona ~ Italy

Glutamate transporter GLT-1 interacts with the three isoforms of α subunit (α1, α2 and α3) of the Na+/K+-ATPase pump yielding a macromolecular complex fundamental for GLT-1-mediated Glu uptake. The distribution of GLT-1 at excitatory synapses is rather well known, whereas few data are available on the detailed expression of α1-3 and, most importantly from a functional perspective, on the quantitative co-distribution of GLT-1 with the different α subunits in intact brain.

Here, we sought to investigate the cellular and subcellular localization of α1-3 and the co-distribution of GLT-1 and α1-3 in excitatory synapses of rat cerebral cortex (SI).

A combination of immunoperoxidase light microscopy, quantitative immuno electron microscopy and multiple labelling confocal microscopy was used to analyze single and multiple labelled intact cortex.

Light microscopy revealed that in all cortical layers α2 is more robustly expressed than α1 and α3. Electron microscopy showed that α1 and α3 are expressed in neuronal elements, whereas α2 was predominantly expressed in astrocytic elements and, occasionally, in neuronal processes. At excitatory synapses, we found that: α1 was rarely localized at postsynaptic dendritic sites and axon terminals; α2 was widely localized at perisynaptic astrocytic processes; and α3 was observed at postsynaptic dendrites and occasionally at axon terminals. Confocal studies showed that GLT-1 was highly co-localized with α2 (~70%), and poorly with α3 (~10%) or α1 (~5%). Analysis of triple labelled sections revealed that GLT-1 co-localized preferentially with α2 or α3 ($P<0.05$) in close relationship with VGLUT1+ terminals.

These results show that in neocortex, α2 is localized in subcellular compartments (e.g., perisynaptic) known to express robustly GLT-1, whereas α1 and α3 are localized in some excitatory axon terminals that are known to express moderately GLT-1. At excitatory synapses, co-distribution of GLT-1 and α2 or α3 isoforms supports a functional coupling between GLT-1 and α isoforms at sites of high demand of Glu uptake.

A MOLECULAR CODE OF NEURAL CONNECTIVITY IN THE PIRIFORM CORTEX

Diodato Assunta, Ruinart De Brimont Marion, Fleischmann Alexander

CIRB- College de France ~ Paris ~ France

The piriform cortex is the largest sub-region of the olfactory cortex and represents one of the higher brain areas in which odor stimuli are thought to be reconstructed to form odor percepts. Odor information encoded by ensembles of piriform neurons must then be transmitted to brain areas involved in the integration of stimuli from different sensory modalities, cognition and motor control. How this connectivity is organized at the cellular level within the piriform cortex remains unknown.

This work aimed to identify the genetic profiles of piriform projection neurons and characterize their spatial organization within the piriform cortex.

We performed RNA deep sequencing of the three piriform layers and characterized layer-specific molecular markers using RNA in situ hybridization and immunohistochemistry. We next performed anterograde neural tracing experiments to identify piriform target regions, and retrograde neural tracing experiments to analyze how piriform projection neurons are organized within piriform cortex.

We show that layers and sub-layers of the piriform cortex can be discriminated by gene expression patterns in adult: for example, Reelin and Fezf2 mark layer IIa, while Cux1 and Npcd mark layers IIb and III. Barhl1, Tle4 and Foxp2 are selectively expressed in subpopulations of cells in layer III. We observe that neurons projecting to distinct target areas are localized in distinct layers. Neurons projecting to the cortical amigdala (CoA) and lateral enthorinal cortex (lENT) are preferentially localized in layer IIa, while neurons projecting to the medial prefrontal cortex (mPFC) are present in layer IIb. Neurons projecting to the olfactory bulb (OB) are localized in layers IIb and III. We then combined the analysis of patterns of gene expression with retrograde tracing experiments to identify molecular signatures of the different subclasses of piriform projecting neurons. Finally, we demonstrate that these molecular signatures of piriform projection neurons are maintained in reeler mice, in which cortical lamination is lost and neural positioning is scrambled, suggesting that piriform output connectivity does not require proper laminar organization.

These results provide important insights into the principles underling the piriform connectivity.

ETHANOL-INDUCED LOSS OF DENDRITIC SPINES IN ACCUMBENS MEDIUM SPINY NEURONS. INSIGHTS AND HINTS FROM A COMPUTATIONAL MODEL

Enrico Paolo[1], Solinas Sergio[1], Spiga Saturnino[2], Giovanna Mulas[2], Diana Marco[3]

[1]Dept. of Biomedical Sciences, University of Sassari ~ Sassari ~ Italy, [2]Dept of Life and Environmental Sciences, University of Cagliari ~ Cagliari ~ Italy, [3]Dept of Chemistry and Pharmacy, University of Sassari ~ Sassari ~ Italy

The nucleus accumbens (Nacc) is a key structure in the neural circuits involved in the control of goal-directed behavior and response to drugs of abuse. Nacc activity is modulated by glutamate-(GLU) and dopamine-(DA) containing projections from cortical and limbic regions respectively, converging on a common postsynaptic target: the medium spiny neuron (MSN).
Ethanol (EtOH) withdrawal profoundly changes the physiology and the morphology of MSNs, with a specific pattern of alterations in both dendritic spine and membrane properties. It has been recently proposed that a close relationship exists between EtOH withdrawal-induced reduction of mesolimbic DA activity and the rearrangement of specific dendritic spines in the Nacc shell MSNs.

Because of their predictive value, computational models are a powerful tool in neurobiological research. We aimed to test whether experimentally observed EtOH withdrawal-induced effects on MSNs could be reproduced in silico. Further we wanted to model the synaptic triad, a particular synaptic architecture characterized by a reciprocal interaction between DA and GLU afferents, typically found on MSN distal dendrites.

We used a Neuron-based biophysically accurate computational model of a Nacc MSN dendrite implementing 3D morphological reconstruction and electrophysiological data. EtOH withdrawal-driven morphological and electrophysiological changes where modeled in order to study the firing rate and discharge pattern of MSNs.

The model findings show that changes in the dendritic spine density and the imbalance in DA/GLU input affect the physiological properties of MSN dendrite, possibly altering its plastic properties.

Biophysically and morphologically accurate computational models could be used to reproduce and study in silico the alterations observed in Nacc MSN physiology during EtOH withdrawal.

Free oral communications session 4

DRUGS OF ABUSE CHANGE NOREPINEPHRINE TRANSMISSION IN THE BED NUCLEUS OF STRIA TERMINALIS (BNST): A MICRODIALYSIS STUDY IN FREELY MOVING RATS

Jadzic Dragana, Mallai Mariangela, Gheza Sara Giulia, Cadeddu Roberto, Carboni Ezio

Dept. of Biomedical Sciences, Univ. of Cagliari ~ Cagliari ~ Italy

The bed nucleus of stria terminalis (BNST) is a limbic brain area included in the extended amygdala. The BNST is innervated by norepinephrine, dopamine and serotonin projections and sends and receives a robust CRF innervation to/from the paraventricular nuclei of hypothalamus. These connections allow the BNST to play a crucial role in the control of the stress response, and in controlling the stressful state associated with drug-seeking that occur after abstinence from chronic drug exposure. We observed earlier that stimulant and non stimulant drugs of abuse, dose dependently increased dopamine extracellular concentration (output) in the BNST suggesting a role for this nucleus in drug addiction.

We investigated here by means of in vivo microdialysis, the acute effect of stimulant and non stimulant drugs of abuse on norepinephrine transmission in the BNST to better understand its role in drug abuse, addiction and relapse.

Norepinephrine and dopamine were assessed in dialysate samples by HPLC and coulometric detection (ESA). Samples were collected (and immediately analysed) every 20 min (flow: 1µL/min) from freely moving rats implanted in the BNST (coordinates: ant. - 0.40; lat. 0.8; vert. 8).

We show here that nicotine (0.2-0.4 mg/kg s.c), morphine (1.0-3.0 mg/kg s.c.), cocaine (2.5-5.0 mg/Kg mg/kg i.p.), kamphetamine (0.5-0.5 mg/kg s.c.) and ethanol (0.5-1.0 mg/kg, i.p.) significantly and dose dependently increased norepinephrine output in the BNST.

These results show that norepinephrine transmission in BNST is involved in the acute effects of drugs of abuse, independently from their stimulant property, therefore suggesting that chronic exposure to drugs of abuse can produce an alteration of norepinephrine transmission that in turn may alter the role that BNST plays in the stress response. In turn stress, by affecting norepinephrine transmission and dopamine transmission in BNST, may play a role in stress-induced drug relapse and in the anxiety/ stress modulation of drug-seeking.

NORADRENERGIC REGULATION OF SPATIAL LEARNING AND MEMORY IN THE RAT: EFFECTS OF SELECTIVE LESIONS AND REPAIR BY TRANSPLANTED NORADRENERGIC NEUROBLASTS

Pintus Roberta, Riggi Margherita, Leanza Giampiero

B.R.A.I.N. Lab for Neurogenesis and Repair, Dept. Life Sciences, University of Trieste, Italy ~ Trieste ~ Italy

Extensive degeneration of noradrenergic (NA) neurons in the Locus Coeruleus/SubCoeruleus (LC/SubC) and loss of fiber terminals in the neocortical and hippocampal target regions are nearly invariant features of Alzheimer's Disease (AD), and are believed to represent early neuropathological events prior to the appearance of overt dementia. However, it is still uncertain whether NA loss is causally linked to cognitive impairments in AD or simply reflects a non-specific response to other insults.

In the present studies, the NA contribution to the regulation of spatial learning and memory was investigated following selective immunolesion of the developing LC/SubC system, followed by bilateral intrahippocampal implantation of a suspension rich in noradrenergic progenitors isolated from the embryonic (13-14 day gestational age) LC.

Starting from 4-6 months and up to about 12 months post-lesion and transplant, the animals underwent sequential tests to evaluate sensory-motor, as well as spatial learning and memory abilities (Morris Water Maze, Radial Arm Water Maze tasks), followed by post-mortem immunohistochemical and stereological analyses.

In no case did the lesion produce sensory-motor changes that would account for performance in the Morris Water Maze task. When tested at about 6 months post-surgery, all animals in the Control, Lesion and Lesion+Transplant groups were equally efficient in the reference memory version of the test, whereas working memory abilities (as assessed by the Radial Arm Water Maze, RAWM, task) were seen consistently impaired in the Lesion-only rats. Interestingly, lesioned animals implanted with reinnervating NA-rich tissue grafts exhibited a fairly normal performance in the RAWM task which, however, became severely impaired following ablation of the transplanted neuroblasts by a further dose of immunotoxin injected bilaterally into the site of implant. Morphological analyses confirmed the massive noradrenergic neuronal and terminal fiber loss induced by the lesion (≥ 90-95%), as well as the near-normal reinnervation of the hippocampus promoted by the transplanted neuroblasts which, however, was completely abolished by the second lesion.

The results suggest that integrity of ascending noradrenergic inputs may be required for the regulation of specific aspects of learning and memory, namely those related to the rapid processing of cognitive information taking place in the hippocampus.

ESCULETIN ATTENUATES LIPOPOLYSACCHARIDE-INDUCED DEPRESSIVE-LIKE BEHAVIOR IN MICE: INHIBITION OF PRO-INFLAMMATORY CYTOKINES, AND UP-REGULATION OF BDNF LEVEL

Sulakhiya Kunjbihari[1], Barua Chandana Chodhury[2], Lahkar Mangala[3]

[1]Laboratory of Neuroscience, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER) ~ Guwahati ~ India, [2]Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara ~ Guwahati ~ India, [3]Department of Pharmacology, Gauhati Medical College ~ Guwahati ~ India

Converging lines of evidence suggest that inflammation, oxidative stress and brain derived neurotrophic factor (BDNF) play important role in the pathophysiology of depression. Esculetin (ESC), a coumarin derived potent antioxidant, is isolated from various plants such as Artemisia scoparia, Artemisia capillaries, Ceratostiggma willmottianum, and Citrus limonia that are used as folk medicines. It is also having antioxidant, anti-inflammatory, anticancer, neuroprotective and hepatoprotective activity.

The main objective of this study was to evaluate the attenuating effect of ESC, in lipopolysaccharide (LPS)-induced depressive-like behaviour and neuro-inflammation in mice.

Mice (n=8/group) were pre-treated with different doses of ESC (25 and 50 mg/kg, p.o) was administered orally for 7 consecutive days, and challenged with saline or LPS (0.83 mg/kg; i.p.) on 8th day. Depressive-like behaviour was assessed by subjecting mice to forced swim test (FST) and tail suspension test (TST) 24 and 28 h post-LPS injection respectively. Animals were sacrificed to evaluate various biochemical parameters in hippocampus such as IL-1β, IL-6, TNF-α, MDA, GSH and BDNF level. In the separate study, 4 h post-LPS injection plasma was collected to measure corticosterone (CORT) level.

Chronic pre-treatment of ESC provided significant ($P<0.05$) protection against LPS-induced reduction in body weight and food intake in mice. High dose of ESC significantly ($P<0.05$) attenuated LPS-induced increase in immobility time in FST and TST. ESC pre-treatment ameliorated LPS-induced neuroinflammation by attenuating hippocampal IL-1β, IL-6, TNF-α level in LPS challenged mice. Moreover, oxidative stress parameters such as MDA and GSH level, and plasma CORT level were significantly ($P<0.05$) normalized by ESC pre-treatment in LPS injected mice. Significant ($P<0.05$) improvement in hippocampal BDNF level was also observed in ESC pre-treated mice challenged with LPS.

Results suggest that ESC attenuated LPS-induced depressive-like behaviour which may be governed by inhibition of cytokines production, oxidative stress, and up-regulation of hippocampal BDNF level. Our result suggests that ESC might be useful in the treatment psychiatric disorders associated with inflammation and oxidative stress.

THE OUTCOME OF ANTIDEPRESSANT TREATMENT ON MOLECULAR, CELLULAR AND BEHAVIORAL ENDOPHENOTYPES OF DEPRESSION IS AFFECTED BY THE QUALITY OF THE LIVING ENVIRONMENT

Poggini Silvia[1], Milior Giampaolo[2], Alboni Silvia[3], Brunello Nicoletta[3], Cirulli Francesca[1], Maggi Laura[2], Branchi Igor[1]

[1]Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome ~ Roma ~ Italy, [3]Department of Life Sciences, University of Modena and Reggio Emilia ~ Modena ~ Italy

Major depression (MD) is a chronic and potentially life-threatening illness that affects up to 10% of the world population. Antidepressant drugs, in particular serotonin selective reuptake inhibitors (SSRIs), represent the standard treatment for MD. However, their efficacy is variable and incomplete.

The aim of this study was to assess the Undirected Susceptibility to Change hypothesis, which posits that SSRIs may not affect mood per se but, enhancing neural plasticity, may render the individual more susceptible to the influence of the environment. Consequently, SSRI administration in a favorable environment would lead to a reduction of symptoms, while in stressful conditions it would worsen the prognosis.

We treated C57BL/6 adult male mice with either fluoxetine (FLX) or vehicle (VEH) for 3 weeks while exposed to either an enriched or a stressful environment, following a chronic stress period (14 days) aimed at inducing a depression-like phenotype. Endophenotypes of MD, including liking- and wanting-type anhedonia, cognitive bias and corticosterone and BDNF levels were assessed. In addition, we investigated hippocampal plasticity, measuring the long term potentiation (LTP) at Schaffer collateral-CA1 synapses.Our results showed that in the enriched environment, FLX mice displayed a significant reduction in wanting-type anhedonia and a higher number of "optimistic" responses in the cognitive bias test, compared to VEH. By contrast, in the stressful condition, FLX mice displayed a significant increase in liking- and wanting-type anhedonia. With regard to the neuroendocrine endpoints, FLX mice exposed to a favorable environment displayed lower corticosterone and higher hippocampal and hypothalamic BDNF levels. Instead, in the stressful condition, FLX mice had a significant increase in corticosterone levels compared to VEH and no difference in BDNF levels was found. Finally, FLX mice showed significantly increased LTP in the stressful but not in the enriched condition. The present findings show that (i) the outcome of fluoxetine treatment depends on the quality of the environment and (ii) fluoxetine amplifies the influence of the environment on the individual, reducing depression-like behavior in a favorable environment but exacerbating it in a stressful condition.

Overall, our results corroborate the Undirected Susceptibility to Change hypothesis, which may be helpful to better understand SSRI action and develop therapeutic strategies aimed at enhancing treatment efficacy through the control of environmental conditions, for instance exploiting cognitive behavioral therapy.

BEHAVIORAL CHARACTERIZATION OF DEHYDROEPIANDROSTERONE EFFECTS IN RATS

Samardzic Janko[1], Svob Strac Dubravka[2], Jadzic Dragana[3], Djuric Milos[1], Obradovic Dragan[1]

[1]Institute of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade ~ Belgrade ~ Serbia, [2]Division of Molecular Medicine, Rudjer Boskovic Institute ~ Zagreb ~ Croatia, [3]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy

Dehydroepiandrosterone (DHEA), a neurosteroid synthesized in the central nervous system from cholesterol, has been associated with various functions in the brain. It appears that the effects of DHEA are primarily mediated through its action on several neurotransmitter systems, including GABA(A), NMDA and Sigma-1 receptors.

The aim of the study was to investigate the behavioral profile of DHEA in the forced swim test (FST) and active avoidance (AA) paradigm, as well as to examine the extent to which GABA(A) receptors contribute to these effects in rats.

In FST, male Wistar rats were forced to swim for 15 min on the first day. Rats were re-exposed to the FST 24 h later for a single 5 min test session, after the acute challenge with saline or DHEA. In AA, the influence of repeated administration of DHEA in rats on the acquisition rate was checked in a procedure lasting five consecutive days. In the experiments, the animals received DHEA (2-50 mg/kg) or saline. Afterwards, the capability of bicuculline (2 mg/kg) to antagonize effects of DHEA was checked. Throughout the study, drugs were given intraperitoneally, 30 min before testing. The data were assessed by one-way ANOVA and Student's t-test. If the ANOVA was significant, each treatment condition was compared with control by Dunnett's test ($\alpha = 0.05$). Where appropriate, the influence of antagonist bicuculline on the effect of DHEA was assessed.

At the dose facilitating retrieval of avoidance memory, DHEA (10 mg/kg) significantly decreased the duration of immobility in FST ($p<0.05$), suggesting antidepressant-like effects. Furthermore, DHEA (10 mg/kg) significantly ($p<0.05$, comparison of regression coefficients by Student's t-test) and progressively increased acquisition rate during 5 days, compared to the saline group. The behavioral effects of DHEA were antagonized by bicuculline (2 mg/kg), a specific antagonist of the GABA(A) receptor.

Our results from FST and AA paradigm experimentally support the findings that under certain circumstances, DHEA produces antidepressant-like and memory-enhancing effects. A recently found correlation between helpless behavior and cognition were fully supported by our results, demonstrating that the antidepressant-like effects of DHEA were obtained at the same dose that induces memory-enhancing effects. Bicuculline abolished the action of DHEA, further suggesting the role for neurosteroids and GABA(A) receptors in the modulation of memory and behavior. However, the neuronal and molecular substrates linking the actions of DHEA to specific GABA(A) receptor complex subunits remain to be further elucidated and linked to human neuropsychiatric disorders.

Free oral communications session 5

NEURITE CONTACT GUIDANCE OF UBIQUITIN LIGASE E3A (UBE3A)-KO NEURONS BY NANO-GROOVED SUBSTRATES

Tonazzini Ilaria[1], Meucci Sandro[2], Van Woerden Geeske[3], Beltram Fabio[2], Elgersma Ype[3], Cecchini Marco[2]

[1]Fondazione Umberto Veronesi, Milano, Italy ~ Milano ~ Italy, [2]NEST, Istituto Nanoscienze-CNR and Scuola Normale Superiore ~ pisa ~ Italy, [3]Department of Neuroscience, Erasmus Medical Center ~ Rotterdam ~ Netherlands

Nano-textured substrates are emerging as tools for investigating and tailoring the processes that regulate neuronal environmental sensing. Nano-engineered substrates are in fact able to induce specific topographical/mechanical stimuli to cells, resembling in vitro several features of extracellular matrix cues, and consequently to tune neuronal differentiation, polarity, migration and neurite orientation.

In the brain, cells are exposed to extracellular stimuli determined by the micro/nano-environment within which they exist, with the local extracellular domains that orchestrate the wiring of the central nervous system. Neurite development is critically controlled by the establishment/maturation of Focal Adhesions (FAs- protein clusters anchoring integrins to cytoskeleton) that in turn coordinate cell polarity and neurite pathfinding/motility.

Although the dynamics of neuronal contact sensing is emerging as crucial for neuronal connectivity and functionality, little is known about these processes in pathological conditions. Nowadays E3 ubiquitin ligases are increasingly recognized as key regulators of neuronal morphogenesis and connectivity. Between these, Ubiquitin E3a ligase (Ube3a) has a key role in brain functioning. The loss of Ube3a expression results in plasticity and learning impairment and recent data suggest that is associated with defects in neuronal structure; however how its loss of function results in neurocognitive impairment, the Angelman Syndrome (AS; OMIN 105830), is still unclear.

Here, the role of Ube3a is investigated in neurite contact guidance during neuronal development. We study the contact guidance of Wild-Type (WT) and Ube3a-KO hippocampal neurons by exploiting nano-grooved substrates with different topographical characteristics with the aim to compare the capability of neurons to read and follow physical directional stimuli.

By exploiting electron beam and nanoimprint lithography we engineered plastic substrates patterned with nanogratings (NGs) having line-widths between 500nm and 1µm. Using these nanotopographies, we studied the molecular processes regulating the interaction between neurons and directional stimuli, as neurite guidance and cytoskeleton organization.

As expected, WT neurons could polarize along the NGs, showing efficient neurite alignment. Interestingly, in Ube3a-KO neurons mechanotransduction was less efficient, as highlighted by an initial loss of cell polarization and neurite alignment. In order to evaluate if this behavior was due to

altered adhesion mechanisms in Ube3a-KO neurons, the activation of FA pathway was investigated, at level of FA-Kinase and Paxillin.

We report that the neuronal contact guidance is less efficient in Ube3a-KO neurons, and this behavior is linked to an impaired activation of FA pathway. Our results suggest that neuronal contact sensing machinery might be affected in AS.

ALGINATE-BASED HYDROGELS FOR CENTRAL NERVOUS SYSTEM TISSUE REGENERATION

Bozza Angela[1], Pongrac Igor[2], Gorup Dunja[2], Alic Ivan[2], Messina Andrea[1], Radmilovic Marin[2], Gajovic Srecko[2], Casarosa Simona[1]

[1]Centre for Integrative Biology - University of Trento ~ Trento ~ Italy, [2]University of Zagreb School of Medicine, Croatian Institute for Brain Research ~ Zagreb ~ Croatia

As the central nervous system (CNS) shows very little capability for self-repair following injury, regenerative medicine approaches are increasingly interested in the use of pluripotent cells or neural stem cells (NSCs) for cell replacement strategies. Biomaterials are an interesting tool to carry out this type of therapies. They are helpful during in vitro differentiation in order to obtain cells at the right developmental stage for transplantation and they could help to enhance and control cell survival after transplantation, minimizing cell death.

We have recently shown that alginate could be a good candidate biomaterial for in vitro neural tissue generation. We now plan to investigate the suitability of alginate as support for neural stem cell injection in the brain.

We encapsulated mouse embryonic stem cells (mESCs) in alginate beads and analyzed by RT-qPCR and immunohistochemistry the efficiency of neural differentiation with respect to two-dimensional control cultures.

We reported alginate ability to support in vitro neural differentiation of encapsulated mESCs. Cells survive inside our scaffolds, showing expression of markers for terminal differentiation and synapses. Cells showed also the capability to form networks among themselves and with cells of other clusters (Bozza et al., Biomaterials 2014).

We will now test feasibility of alginate injections in the brain tissue, its in vivo crosslinking after injection and the levels of inflammation due to its presence in mouse brain tissue. Histological preliminary results suggest that a hydrogel forms in the tissue. Moreover, bioluminescence analyses in TLR2-luciferase mice suggest that it does not elicit inflammatory response following injection. We plan to co-inject alginate with NSCs in mouse models of brain injury. We plan to study alginate permanence in the brain and NSCs viability, integration and capability to stimulate regeneration after ischemia.

We have set up a novel three-dimensional culture system bsed on alginate encapsulation that allows for efficient neural differentiation. Our in vivo preliminary results suggest that alginate could also be suitable for in vivo approaches.

GENETIC ABLATION OF HOMEODOMAIN INTERACTING PROTEIN KINASE 2 (HIPK2) SELECTIVELY INDUCES APOPTOSIS OF CEREBELLAR PURKINJE CELLS AND GENERATES AN ATAXIC-LIKE PHENOTYPE

Anzilotti Serenella[1], Tornincasa Mara[2], Gerlini Raffaele[2], Conte Andrea[2], Brancaccio Paola[3], Cuomo Ornella[3], Fusco Alfredo[2], Annunziato Lucio[3], Pierantoni Giovanna Maria[2], Pignataro Giuseppe[3]

[1]SDN IRCCS ~ Naples ~ Italy, [2]Institute of Endocrinology and Experimental Oncology of National Research Council and Department of Molecular Medicine and Medical Biotechnology, School of Medicine, "Federico II ~ Naples ~ Italy, [3]Division Pharmacology, Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II ~ Naples ~ Italy

Homeodomain interacting protein kinase 2 (HIPK2) is a multitalented coregulator of an increasing number of transcription factors and cofactors involved in cell death and proliferation in several organs and systems.

Since Hipk2-/- mice show behavioral abnormalities consistent with cerebellar dysfunction, we investigated whether Hipk2 is involved in these neurological symptoms.

We characterized the post-natal developmental expression profile of Hipk2 in the brain cortex, hippocampus, striatum, and cerebellum of mice by real time PCR, western blot analysis, and immunohistochemistry. Then, we have carried out behavioral experiments in order to establish the role of HIPK2 in several brain functions.

We found that whereas in the brain cortex, hippocampus, and striatum HIPK2 expression progressively decreased with age, i.e., from post-natal day 1 to adulthood, it increased in the cerebellum. Interestingly, mice lacking Hipk2 displayed atrophic lobules and a visibly smaller cerebellum than did wild-type mice. More important, the cerebellum of Hipk2-/- mice showed a strong reduction in cerebellar Purkinje neurons. Such reduction is due to the activation of an apoptotic process associated with a compromised ubiquitin-mediated proteosomal degradation of beta-catenin. In particular, Purkinje cell dysfunction was characterized by a strong interaction between non phosphorylated beta-catenin and ubiquitin, followed by an unpredicted beta-catenin accumulation. Moreover, our behavioral tests showed that Hipk2-/- mice displayed muscle and balance impairment, indicative of Hipk2 involvement in cerebellar function.

Taken together, these results indicate that Hipk2 exerts a relevant role in the survival of cerebellar Purkinje cells and that Hipk2 genetic ablation generates cerebellar dysfunction compatible with an ataxic-like phenotype.

MICROGLIAL MICROVESICLES AS THERAPEUTIC VECTOR FOR NEUROINFLAMMATION

Casella Giacomo[1], Colombo Federico[1], Finardi Annmaria[2], Furlan Roberto[2]

[1]Università Vita e Salute ~ Milano ~ Italy, [2]Ospedale San Raffaele ~ Milano ~ Italy

Extracellular vesicles EVs are membrane-bound particles formed from inside a cell or directly from its membrane and released to the extracellular space that carry information whose function is cell-to-cell communication without direct contact. EVs can be divided by their biogenesis, cell origin and morphologic characteristics, in to three classes: exosomes, shedding vesicles SVs and apoptotic blebs. All most of cells release EVs.

We propose here to exploit microglia-derived EVs as drug delivery tool for neuroinflammation and neurodegeneration, trough production of microglial SVs able to cross the blood brain barrier BBB and deliver therapeutic molecules to the central nervous system CNS. We have produced a stably engineer murine microglia cell lines to express interleukin 4. Our interest is direct to use IL-4, because it can shift microglia to an alternative phenotype called M2.

We are trying to pseudotyping these SVs with the aim to target the microglia in the CNS; for this purpose, we are testing different molecules e.g: the Rabies viral glycoprotein RVG, which has a high neurotrophism and different “eat signals” able to promote the uptake of the SVs by the phagocytosisWe optimized the microglial SVs production using ATP, PMA, and the collection by different step of centrifugation. We evaluated if the SVs can transfer their content in vitro using the farnesylate GFP; another approach that we are testing is the CRE- lacZ+ model.

MVs IL-4+ have showed the ability to promote the polarization of the microglia in vitro by the expression of a typical anti-inflammatory gene like YM1, Arg1 and induce a slight reduction of pro-inflammatory gene iNOS.

Our preliminary results seem to suggest the efficacy of our engineered SVs molecular profile in delivery the IL-4 response in to recipient cells in vitro. This should be a starter point from which to test therapeutic efficacy of this method.

NON-PARALYTIC BOTULINUM MOLECULES FOR THE CONTROL OF PAIN

Mangione Antonina Stefania[7], Obara Ilona[2], Maiarú Maria[1], Geranton Sandrine Martine[1], Blandini Fabio[4], Tassorelli Cristina[3], Ferrari Enrico[5], Davleton Bazbek[6], Hunt Stephen Philip[1]

[1]Cell and Developmental Biology, University College London ~ London ~ United Kingdom, [2]Sch. of Med., Pharmacy and Health., Durham Univ. ~ Stockton-on-Tees ~ United Kingdom, [3]Laboratory of Neurophysiology of Integrative Autonomic System, Headache Sci. Ctr., "C. Mondino" Natl. Neurological Inst. ~ Pavia ~ Italy, [4]Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [5]Sch. Of Life Sci., Univ. of Lincoln ~ Lincoln ~ United Kingdom, [6]Dept. of BioMed. Sci., Univ. of Sheffield ~ Sheffield ~ United Kingdom, [7]Cell and Developmental Biology, University College London (London, United Kingdom); Laboratory of Neurophysiology of Integrative Autonomic System, Headache Sci. Ctr., "C. Mondino" Natl. Neurological Inst. ~ Pavia ~ Italy

Local injections of Botulinum toxin A (BoNT/A) have been reported to be useful for the treatment of painful disorders, including migraine, diabetic neuropathy, postherpetic neuralgia and myofascial pain. BoNT/A blocks the release of inflammatory pain mediators (SP and CGRP) via cleavage of SNAP-25, a SNARE protein essential for neuroexocytosis. However, BoNT/A also causes muscle paralysis by blocking release of transmitter at the neuromuscular junction. Recently, Davletov and colleagues developed a non-paralytic botulinum based molecule (Bitox) that exhibited the same 'silencing' efficacy on central neurons as the native BoNT/A but had significantly reduced paralytic activity.

We examined whether Bitox delivered peripherally was able to mimic the reported actions of BoNT/A by 1) blocking local plasma extravasation from nociceptive C fibers and 2) reducing nociceptor activity in animal models of chronic pain and inflammation.

Rats received intraplantar injection of Bitox (200ng/50µl saline) or vehicle 1) 14 days before or 72 h after spared nerve injury (SNI), 2) 72h before capsaicin or formalin injection, 3) 24 and 72h after complete Freund's adjuvant (CFA) injection in ankle joint or plantar surface of the hind paw or 4) 24h after plantar incisional injury. Mechanical thresholds were measured with von Frey hairs and thermal thresholds were assessed with Hargreaves apparatus. Pin-prick test was performed in the lateral area to assess secondary hyperalgesia after plantar capsaicin injection into the central area of the plantar surface. In addition, capsaicin-induced plasma extravasation and 5-bromo-2'-deoxyuridine (BrdU) incorporation after Bitox intraplantar injection were evaluated.

No motor deficits were seen and acute nociceptive thresholds were unimpaired by Bitox injections. Nociceptive thresholds increased only when A-nociceptor function was implicated, for example, in neuropathic pain models and secondary hyperalgesia associated with either CFA-induced arthritis or capsaicin injection. Inflammatory and incisional pain hypersensitivity mediated primarily by C-nociceptors was not reduced by Bitox injections. Bitox reduced the extravasation induced by capsaicin-sensitive C-nociceptors as well as the number of BrdU-incorporated cells in the skin 30 days after local Bitox injection, suggesting that Bitox influences keratinocytes proliferation by inhibiting the neurotransmitters release in the skin.

We show that Bitox, a non-paralysing version of BoNT/A, reduces A-nociceptor but not C-nociceptor activity in rat pain models. These results, taken together with recent clinical data, suggest that the use of BoNT/A or Bitox should be targeted specifically to pain conditions in which A-nociceptors are thought to play a significant role. Supported by MRC grant MR/K022539/1.

Free oral communications session 6

EMX2 ROLE IN CORTICOCEREBRAL ASTROGENESIS AND GLIOBLASTOMA TUMORS

Falcone Carmen[1], Filippis Carol[1], Granzotto Marilena[2], Daga Antonio[3], Sgubin Donatella[5], Leanza Giampiero[4], Mallamaci Antonello[1]

[1]SISSA ~ Trieste ~ Italy, [2]Azienda Ospedaliera Ospedali Riuniti ~ Trieste ~ Italy, [3]IRCCS AOU San Martino IST ~ Genova ~ Italy, [4]Università di Trieste ~ Trieste ~ Italy, [5]Unità Operativa di Neurochirurgia, Istituto Clinico Città Studi ~ Milano ~ Italy

Cortico-cerebral astrocytogenesis is a tightly regulated process. It initiates at low level in the middle of neuronogenesis and peaks up after its completion. Astrocytic outputs depend on two primary factors: progression of multipotent precursors towards the astroglial lineage and sizing of the astrogenic proliferating pool. The proper sizing of astrocytic compartment is crucial for the adult brain functions. Uncontrolled proliferation of astroglial cells in adult may give rise to severe pathologies, such as glioblastoma multiforme, one of the most aggressive malignant tumors in humans.

We studied the role of the Emx2 gene in the fate choice of cortical neural stem cells as well as in the proper regulation of astroblasts proliferation. Also, we investigated whether the modulation of Emx2 expression levels could be exploited for therapeutic applications, in particular in the suppression of glioblastoma tumors.

As for the study of cortico-cerebral astrogenesis, we used combined gain- and loss-of-function methods. Tests were run in vivo as well as in primary cultures of cortico-cerebral precursors. We investigated the impact of Emx2 on glioblastoma by using in vitro cultures of malignant patient cells.

We found that Emx2 overexpression in cortico-cerebral stem cells shrinked the proliferating astrogenic pool, resulting in a severe reduction of the astroglial outcome. We showed that this was caused by EgfR and Fgf9 downregulation and that both phenomena originated from exaggerated Bmp signalling and Sox2 repression. Finally, we provided evidence that in vivo temporal progression of Emx2 levels in cortico-cerebral multipotent precursors contributes to confine the bulk of astrogenesis to postnatal life. Interestingly, we discovered that Emx2 is able to reduce the proliferation of glioblastorna tumor cells. We are currently investigating the molecular mechanisms underlying Emx2 activity in glioblastorna tumors.

Emx2 regulation of astrogenesis adds to a number of earlier developmental processes mastered by this gene. It points to Emx2 as a new promising tool for controlling a variety of pathological processes, including glioblastoma, and optimizing cell-based designs for brain repair.

THE CROSSTALK WITHIN THETA RHYTHMS BETWEEN SECONDARY AUDITORY CORTEX AND BASOLATERAL AMYGDALA IS ESSENTIAL DURING REMOTE FEAR MEMORY RECALL

Cambiaghi Marco[1], Grosso Anna[1], Likthtik Ekaterina[2], Mazziotti Raffaele[3], Concina Giulia[1], Renna Annamaria[1], Sacco Tiziana[1], Gordon Joshua[2], Sacchetti Benedetto[1]

[1]Levi-Montalcini Department of Neuroscience, University of Turin ~ Torino ~ Italy, [2]Department of Psychiatry, College of Physicians and Surgeons, Columbia University ~ New York ~ United States, [3]Institute of Neuroscience, CNR ~ Pisa ~ Italy

Negative experiences are quickly learned and long remembered. The secondary auditory cortex (Te2) and the basolateral amygdala (BLA) are both involved in long-term fear memory. Indeed, in auditory fear conditioned rats, secondary auditory cortex is essential for encoding the emotional valence acquired by the auditory stimuli at remote time points. Brain oscillations, particularly the theta rhythm (4-12 Hz), seem to play a crucial role in the memory coding process and connections with amygdala.

The present study examined neural activity between Te2 and BLA during the recall of recent and remote fear memories.

To this end, we obtained LFP and multi-unit activity (MUA) recordings in Te2 and BLA of rats that underwent recall at 24 hours and 30 days after the association of an acoustic conditioned (CS, tone) and an aversive unconditioned stimulus (US, electric shock).

Power spectral analysis of Te2 activity during the recall of aversive memories showed modality-specific significant changes in the theta band, at both 24h and 30 days. In particular, whereas low-theta (3-7 Hz) power increased in both conditions, high-theta (7-12 Hz) power decreased at both recent and remote retrieval. Remote memory recall was also associated with a modality and region specific increase in Te2-BLA low-theta synchrony. Furthermore, MUA recordings confirmed that BLA synchrony with the Te2 correlates with better memory at the remote time-point.

Our study demonstrates the functional involvement of Te2 in the expression of auditory fear memory at remote time point.

IN VITRO GLUTAMATE TREATMENTS MODIFY AMPA RNA EDITING AND ADARS ACTIVITY

Filippini Alice, Bonini Daniela, La Via Luca, Gardella Rita, Colombi Marina, Barbon Alessandro

Università degli Studi di Brescia ~ Brescia ~ Italy

Adenosine-to-inosine RNA editing is a post-transcriptional process, catalyzed by ADAR enzymes, with an important role in diversifying the number of proteins derived from a single gene. In neurons, editing of ionotropic AMPA glutamate receptors has been shown to be altered under several experimental conditions, including severe pathologies, thus highlighting the potential significance of its modulation; moreover glutamate receptors excessive stimulation might lead to a dramatic influx of Ca2+ in neuron with the activation of a series of detrimental events leading to cell death.

In this study we focused our attention on RNA editing and ADAR activity, in order to understand some of the mechanisms which might be involved in protecting neurons from excitotoxicity.

We treated rat primary cortical cell cultures with a sub-lethal dose of glutamate (10 µM) for 24 hours.
After treatment, cells were maintained in normal culture medium for an additional 3 d (washout). BAPTA/am intracellular calcium chelator 20µM and Calpain inhibitor 10 µM were added 30 min prior to addition of Glutamate and maintained for the 24 h of chronic treatment.
The editing level for the target genes was quantified by sequence analysis.

We found that chronic glutamate treatment down-regulates RNA editing levels at the R/G site of GluA2-4 subunits of AMPA receptors and at the K/E site of CYFIP2. These changes are site-specific since they were not observed either for the GluA2 Q/R site or for other non-glutamatergic sites. Glutamate treatment also down-regulates the protein expression levels of both ADAR1 and ADAR2 enzymes, through a pathway that is Ca2+- and calpain-dependent.

Given that AMPA receptors containing unedited subunits show a slower recovery rate from desensitization compared to those containing edited forms, the reduced editing at the R/G site may, at least in part, compensate for glutamate over-stimulation, perhaps through the reduced activation of postsynaptic receptors. In summary, our data provide direct evidence of the involvement of ADAR1 and ADAR2 activity as a possible compensatory mechanism for neuronal protection following glutamate over-stimulation.

DELETION OF LRRK2 PROTECTS MICE FROM EARLY COGNITIVE IMPAIRMENTS INDUCED BY ALPHA-SYNUCLEIN FIBRIL INOCULATION

Volta Mattia, Bergeron Sabrina, Mitchell Emma, Cataldi Stefano, Macisaac Sarah, Beccano--Kelly Dayne, Hicks Andrew, Pramstaller Peter, Milnerwood Austen, Farrer Matthew

Center for Biomedicine, EURAC Research

Exogenous pathological forms of alpha-synuclein (aSyn) have been reported to seed the formation of intracellular aggregates of endogenous soluble aSyn in vitro and in vivo. Injection of recombinant aSyn fibrils or brain homogenate from symptomatic A53T-aSyn overexpressing mice (exhibiting widespread synucleinopathy) induces progressive neuropathology, neurodegeneration and behavioral alterations in otherwise healthy wild-type animals. Genetic variability and mutations in the SNCA and LRRK2 genes are separately the main causes of familial Parkinson's disease (PD).

The proteins are reported to interact physiologically and co-localize in Lewy body aggregates, suggesting a potential relationship. While one report demonstrated that LRRK2 overexpression accelerated mutant aSyn-induced pathology, others showed little or no additive effects in overexpressor mouse double-crosses. Thus, the synergistic pathological relationship between LRRK2 and aSyn remains unclear. We sought to investigate this question while avoiding potential complications arising from exaggerated protein levels and potentially altered expression patterns. We treated cortical neuron enriched cultures prepared from wild-type (WT) and LRRK2 KO mice with either aSyn pre-formed fibrils (PFFs) or PBS vehicle. Immunocytochemical analysis showed stark pathological pSer129-aSyn-positive aggregates in WT neuron cultures treated with PFF. The density of staining and aggregates was lower in cultures from LRRK2 KO littermates. To explore the protective effect of LRRK2 deletion in vivo we unilaterally injected the dorsolateral striatum of WT and LRRK2 KO mice with PFFs or PBS. Animals were assessed before, 30, 90, 180 and 270 days post injection (dpi) in behavioral paradigms assessing locomotion, motor symmetry, anxiety and cognition. We observed a rapid (30dpi) recognition memory defect in PFF-treated WT mice, whereas KO animals were protected from PFF-induced cognitive impairments up to 270dpi. In addition, PFFs injection induced asymmetry of forelimb stepping in WT mice, but no KO, at the same time-points. Administration of monomeric aSyn in a separate cohort of WT mice had no behavioral consequences at 30dpi. Critically, acute silencing of LRRK2 in the brain rescued the early cognitive deficits induced by striatal PFFs.

Mice were then transcardially perfused and neuropathology assayed by pSer129-aSyn staining. Notably, no aggregation was detected at the timepoint at which cognitive impairments were first observed (30dpi). Behavioral deficits persisted and mild neuropathology was eventually observed at 90dpi, although no significant differences were found between genotypes.
It has been proposed that a lack of LRRK2 protects against viral-mediated aSyn toxicity via a reduced immune response. Therefore, we assessed Iba1-positive microglia staining in the striatum and cortex of PFF-treated animals. Iba1 staining was comparable in all experimental groups, in all regions analyzed, at 30 and 90dpi.

The data expand our current understanding of the consequences of amyloid-like protein insults and provide evidence that LRRK2 and aSyn interactions contribute to neuropathology and behavioral

deficits induced by aSyn PFFs. As genetic LRRK2 deletion was protective against PFF-induced aggregation in neuronal cultures, in which glia are

absent, the data suggest that PFF insults and LRRK2 interactions occur in a purely neuronal setting. This conclusion is supported by the absence of microgliosis in vivo. Importantly we observe a disconnect between neuropathology and cognitive impairments, suggesting PFF treatment leads to pathophysiological processes that precede, or are entirely independent of, protein aggregation. These data will form the basis for more comprehensive investigations of the molecular mechanisms underlying synucleinopathies and familial parkinsonism.

LONG LASTING EFFECTS OF ADOLESCENT MDMA AND CAFFEINE EXPOSURE ON MESOLIMBIC DOPAMINE TRANSMISSION, COGNITIVE FUNCTION AND DOPAMINERGIC NEUROTOXICITY IN RATS

Cadoni Cristina[1], Pisanu Augusta[1], Simola Nicola[4], Corongiu Silvia[4], Dessì Christian[1], Frau Lucia[2], Porceddu Francesca[4], Sil Annesha[2], Wardas Jadwiga[3], Di Chiara Gaetano[4]

[1]Institute of Neuroscience, National Research Council of Italy ~ Cagliari ~ Italy, [2]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [3]Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences ~ Krakow ~ Poland, [4]Department of Biomedical Sciences, Neuropsychopharmacology Section, University of Cagliari ~ Cagliari ~ Italy

After a period of decline the use of MDMA (3,4-methylendioxymethamphetamine, ecstasy) is now recovering and this is particularly alarming since most of users are adolescents. The fact that MDMA is frequently taken with other substances, among these being caffeine, posits an urgent need to investigate possible synergic effects. While MDMA neurotoxicity on serotonin (5-HT) neurons is well established, both in laboratory animals and in humans, neurotoxicity on dopamine (DA) neurons is still debated. Although extensive evidence indicates a key role of DA in stimulant, rewarding and many acute toxic effects of MDMA, research on DA involvement in the long term effects of MDMA has been overlooked.

The present study is aimed to shed light on the long-term effects of repeated adolescent exposure to MDMA alone and combined with caffeine on mesolimbic DA transmission, cognitive and emotional functions at adulthood, and to ascertain possible neurotoxic effects on DA neurons.

Male adolescent Sprague-Dawley rats were exposed to MDMA (5 mg/kg s.c.), MDMA + caffeine (5 mg/kg + 10 mg/kg i.p.) or vehicle once a day for 10 days (on days 1-5 and 8-12). At adulthood animals underwent microdialysis experiments in the nucleus accumbens (NAc) shell and core. Other groups of rats were tested for cognitive and emotional impairment (NORT and EPM). To investigate neurotoxic effects on DA neurons TH-immunohystochemistry analysis in striatum and NAc shell and core and stereological counts of TH-positive neurons in VTA and SNC were performed.

Significant reduction of DA basal values were observed in the NAc core of MDMA exposed rats. Adolescent exposure to MDMA induced behavioral sensitization to MDMA at adulthood associated with a potentiation of DA response in the NAc core. Caffeine co-administration did not change this effect. In the NAc shell no differences were observed in the DA stimulating effects of MDMA between groups. Animals exposed to MDMA and MDMA + caffeine displayed cognitive deficits but no change in anxiety. Moreover a reduced density of TH-immunostaining was observed in the striatum, NAc shell and core of both MDMA groups, as well as a reduced density of TH-positive neurons in VTA and SNC.

Our results while confirming the ability of MDMA to induce behavioral and biochemical sensitization, not affected by caffeine, demonstrate a long-lasting impairment of cognitive functions and suggest a neurotoxic effect of MDMA also on DA neurons as well being neurotoxic for 5-HT neurons.

Free oral communications session 7

EFFECT OF CURCUMINS IN RNA REGULATION AND IN AMYLOID BETA AGGREGATION IN ALZHEIMER'S DISEASE PATIENTS

Gagliardi Stella[2], Ryan Daniel[1], Sorrentino Stefano[2], Policicchio Stefania[2], Sinforiani Elena[3], Bini Paola[4], Cashman John[1], Cereda Cristina[2]

[1]Laboratory of Chemistry, Human BioMolecular Research Institute ~ San Diego ~ United States, [2]Laboratory of Experimental Neurobiology, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [3]Laboratory of Neuropsychology/UVA, C. Mondino National Neurological Institute ~ Pavia ~ Italy, [4]Division of General Neurology, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy

Introduction. The natural product mixture of curcuminoids is of particular interest, as they may selectively enhance ABeta phagocytosis, attenuate APP maturation and alter gene transcription in blood cells of Alzheimer's disease (AD) patients. (Fiala et al., 2007; Gagliardi et al., 2012). Different compound have been tested and one of the most potent was bisdesmethoxycurcumin (BDC).

Genes expression and immunofluorescence studies in peripheral blood mononuclear cell (PBMC) of AD patients in response to curcumins treatment.

We analyzed 20 PBMC samples from AD patients and 20 controls treated with Abeta and BDC to test the RNA expression of beta1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl transferase (MGAT3) and vitamin D receptor (VDR) genes by Real Time PCR. The same samples have been used for immunofluorescence experiments to morphologically evaluate the sub-cellular distribution and appearance of Abeta. Moreover we tested two other curcuminoid compounds (29 and 18) that have been described to be active in AD cellular model (Gagliardi et al., 2012).

Our data showed that compared to controls, MGAT3 and VDR mRNA levels were up-regulated in PBMC from AD patients treated with Abeta and BDC compare to samples treated only with Abeta. Immunofluorescence showed the reduction of Abeta aggregates in AD patients treated with BDC compared to the samples not treated. Preliminary data showed that Compound 29* may also stimolate Abeta reduction. About Compound 18*, it seems to be not active in Abeta accumulation.

We demonstrated that BDC treatment may impact both gene expression and Abeta phagocytosis. MGAT3 is a gene essential for phagocytic functions and the over-expression of VDR suppressed amyloid precursor protein (APP) transcription in neuroblastoma cells (Wang et al., 2012). This preliminary data suggest a proof of concept for a future pharmacological intervention using curcumins. Work in progress: More curcuminoid compounds have been test by RNA-seq (Illumina) to determinate the RNA regulation in response to curcumins. The data are being analyzed by Bioinformatic team.

IDENTIFICATION OF MICRORNAS POTENTIALLY REGULATING THE EXPRESSION OF GPR17, A RECEPTOR INVOLVED IN THE DEVELOPMENT OF OLIGODENDROGLIAL PRECURSOR CELLS

Marangon Davide[1], Coppolino Giusy Tindara[1], Finardi Annamaria[2], Furlan Roberto[2], Abbracchio Maria Pia[1], Lecca Davide[1]

[1]Laboratory of Molecular and Cellular Pharmacology of the Purinergic Transmission, Department of Pharmacological and Biomolecular Sciences, University of Milano ~ Milano ~ Italy, [2]Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute ~ Milano ~ Italy

GPR17 is a G protein-coupled receptor physiologically expressed in oligodendroglial precursor cells (OPCs). The receptor is necessary in the early stages of differentiation, but then it has to be down-regulated to allow terminal maturation. Any alteration in its expression pattern leads to delay in maturation, suggesting the importance of this receptor as a checkpoint during differentiation.

This work was aimed at assessing if the expression of GPR17 can be regulated at post-transcriptional level by microRNAs.

OPCs were cultured in presence of growth factors for 2-3 days and then they were switched to a differentiating medium. At the same time cells were transfected with miRNA mimics or inhibitors at the final concentration of 50 nM. Two days post-transfection, cultures were fixed or harvested for either immunocytochemistry or qRT-PCR analysis.

We analyzed GPR17 3'-UTR in silico with six different prediction tools and we identified a miRNA candidate (that we named miR-X). Interestingly, we found that overexpression of this miRNA in cultured OPCs differentiated with T3 strongly reduced the number of mature oligodendrocytes expressing MBP, whereas, the number of GPR17-positive cells was not affected. However, the transfection of miR-X in milder differentiating conditions (absence of T3) caused a strong reduction in the number of GPR17-positive cells, suggesting that this miRNA can also repress OPC differentiation at earlier stages. A luciferase reporter assay on the GPR17 3'UTR revealed that miR-X was not able to decrease the luciferase activity, suggesting that the observed effects on GPR17 expression are not mediated by direct binding of miR-X to GPR17 mRNA. Levels of miR-X are altered in cerebrospinal fluid of multiple sclerosis patients, suggesting that it could be a useful biomarker of disease. Taking advantage of Ingenuity Pathway Analysis tool, we have built a model network connecting already validated targets of miR-X (e.g. protein kinases, cytoskeletal proteins and neuregulins) to the expression of myelin genes. We are now working on this network to prove that miR-X can lead to a decrease in MBP expression and to a delay in oligodendroglial maturation through the simultaneous inhibition of several targets.

Taken together, these results suggest that miR-X synergically inhibits different target genes involved in myelination, such as GPR17 and MBP. Integration of the mechanisms connecting all the validated targets of this miRNA could shed light on a very complex and redundant regulatory network.

Sponsored by FISM 2013/R-1 project to MPA and by Fondazione Cariplo, grant n° 2014-1207 to DL.

EXOSOMES FROM MESENCHYMAL STEM CELLS: EXPERIMENTAL ASSESSMENT ON IN VITRO AND IN VIVO MODELS OF ALS

Bonafede Roberta, Scambi Ilaria, Schiaffino Lorenzo, Mariotti Raffaella

University of Verona ~ Verona ~ Italy

Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disease and mutations in superoxide dismutase gene (SOD1) are the major genetic contributor to ALS. Therapeutic strategies for ALS are actually minimally effective on patients' survival and quality of life. Stem cells represent a promising therapeutic approach in the treatment of neurodegenerative diseases and their beneficial effect seem to be due through a paracrine effect via the release of exosomes.

In the present study we wanted to assess the neuroprotective effect of exosomes derived from adipose-derived stromal cells (ASC) on in vitro and in vivo models of ALS.

Murine ASC were isolated from inguinal adipose tissues of C57BL/6 mice; exosomes were obtained from ASC supernatants and were identified by electron microscopy and Western blot. Motoneuron-like cell line (NSC-34) naïve and overexpressing different human SOD1 mutants (G93A, G37R, A4V) were used as an in vitro models of ALS to evaluate the possible protective effect of exosomes after oxidative insult (H_2O_2). Cell death were evaluated with acridine orange/propidium iodide double staining. For in vivo experiments, transgenic mice overexpressing mutant human SOD1(G93A) gene were used. We injected mice intravenously with ASC-exosomes at clinical onset until terminal stage, once a week, to assess whether systemic administration of ASC-exosomes can ameliorate the disease course and lifespan. In parallel, facial nerve crush was used as a model of neurodegeneration and exosomes were injected in the whisker pad on the lesion side of the snout to evaluate, by immunohistochemical investigation of the facial motor nucleus, the effect of exosomes in microglia response.

In "*in vitro"* experiments, the administration of ADSC-exosomes on NSC-34 cells naïve and stably transfected with all different human mutant SOD1 gene protected cells from oxidative damage, with a significantly increase of cell viability. In in vivo experiments on SOD1(G93A) mice the evaluation of motor performance and lifespan between exosomes-treated and control animals is ongoing. On the other hand, the exosomes exert a beneficial effect on crushed facial motoneuron decreasing glial activation.

ASC-exosomes could represents a promising therapeutic approach in neurodegenerative disorders.

SAFFRON TREATMENT IN RAT MODEL OF RETINAL DYSTROPHY

Di Paolo Mattia, Maccarone Rita, Bisti Silvia

Department of Biotechnology and Applied Clinical Science ~ l'Aquila ~ Italy

Retinal neurodegenerative diseases are a group of clinically and genetically heterogeneous disorders characterized by progressive loss of vision due to neuronal death. Nowadays it is still missing an effective treatment for these pathologies and it is only possible to mitigate and eventually stop the progress of the neurodegeneration using newly discovered neuroprotective molecules. Natural compounds are today considered a useful remedy against many forms of neurodegenerations and our lab has pioneered the use of the saffron, a natural spice, to mitigate oxidative stress and neuro-inflammation in retinal neurodegenerations such as the Age-related Macular Degeneration (AMD).

In this work we tested the same protective agent, saffron, in a different animal model: the Royal College of Surgeon rats (RCS/lav; with a defect in the retinal dystrophy gene, rdy) suffer of an inherited retinal degeneration that mimics a form of human retinitis pigmentosa.

To investigate the effects of saffron, we divided animals into three experimental groups: 1) RCS/lav as dystrophic control 2) RCS/lav + Saffron 3) RCS/lav-rdy, rats homozygous for the normal rdy allele, as non-dystrophic congenic control. Animals treated with Saffron were fed from fetal age, indirectly, until the sacrifice.In order to evaluate the degree of damage in the different stages of retina development we monitored treated and untreated retinae using Elettroretinogram (ERG) and Flicker ERG recording as functional techniques and retinal thickness, cellular death, inflammatory and trophic markes (GFAP, FGF) in the morphological analysis .

It was observed a significant improvement on the early ERG response and an important influence on the rate of photoreceptor death of treated group respect to the control. Available data suggest that saffron effect doesn’t involve Flicker ERG function and others morphological features.

Results show that saffron treatment can positively modulate some aspects of the retinal pathogenes slowing down, slightly, neurodegenerative progression.

INTRACAROTID INFUSION OF MESENCHYMAL STEM CELLS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE, FOCUSING ON CELL DISTRIBUTION, NEUROPROTECTIVE AND BEHAVIORAL EFFECTS

Cerri Silvia[1], Greco Rosaria[2], Levandis Giovanna[1], Ghezzi Cristina[1], Mangione Antonina Stefania[2], Fuzzati Armentero Marie Therese[1], Bonizzi Arianna[1], Avanzini Maria Antonietta[3], Maccario Rita[3], Blandini Fabio[1]

[1]Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [2]Laboratory of Neurophysiology of Integrative Autonomic Systems, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [3]Immunology and Transplantation Laboratory/Cell Factory/Pediatric Hematology/Oncology Department, Fondazione IRCCS Policlinico San Matteo ~ Pavia ~ Italy

Mesenchymal stem cells (MSCs) have been proposed as a potential therapeutic tool for Parkinson's disease (PD) and systemic administration of these cells has been tested in both pre-clinical and clinical studies. However, no information on survival and actual capacity of MSCs to reach the brain has been provided.

In this study, we firstly evaluated homing of intraarterially infused rat (r)MSCs in the brain of rats bearing a 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal tract, to establish whether the toxin-induced damage is sufficient to grant MSC passage across the blood-brain barrier (BBB), or a transient BBB disruption is necessary. Moreover, the rMSC distribution in peripheral organs and the effects of cell infusion on neurodegenerative process and motor deficits were investigated.

Rat MSCs were infused fourteen days after 6-OHDA injection. Hyperosmolar solution of mannitol was used to transiently permeabilize the BBB. Motor impairment was assessed by adjusting step test (forelimb akinesia) and rotational response to dopaminergic stimulation (apomorphine). Seven and twenty-eight days after cell infusion, animals were sacrificed and lesions in the striatum and substantia nigra were evaluated. Immunohistochemical analysis of striatal c-Fos expression was performed to evaluate rMSC-induced changes in the functional response of striatal neurons to apomorphine.

An appreciable delivery of rMSCs to the brain of 6-OHDA lesioned animals was obtained only after mannitol pre-treatment. A notable percentage of infused cells accumulated in peripheral organs. Infusion of rMSCs did not modify the progression of 6-OHDA-induced damage or the motor impairment at the stepping test, but induced progressive normalization of the pathological response (contralateral turning) to apomorphine administration. The disappearance of the turning behavior in rMSC-treated lesioned animals, at the fourth week post-infusion, was associated with increased c-Fos expression in the intact striatum, while no changes were detected in the lesioned side in comparison with rMSC-untreated lesioned animals.

BBB permeabilization is required to grant passage of rMSCs into the brain. MSC infusion did not modify the progression of PD-like neuronal damage, but profoundly affected behavioral stereotypies triggered by apomorphine. This suggests that arterially-infused MSCs may induce functional compensatory changes in the nigrostriatal system by modulating the responsiveness of striatal neurons to dopaminergic stimulation. Future investigations should further explore this

capability of MSCs, while neuroprotective or neurorestorative effects – at least under these experimental conditions – can be excluded.

POSTERS

P 1/09

NF-KAPPAB P50 KNOCK-OUT MICE AS ANIMAL MODEL OF NEURODEVELOPMENTAL DISORDERS

Bonini Sara Anna, Mastinu Andrea, Maccarinelli Giuseppina, Premoli Marika, Ferrari--Toninelli Giulia, Memo Maurizio

Department of Molecular and Translational Medicine, University of Brescia ~ Brescia ~ Italy

Alterations in genes that regulate neurodevelopment can lead to cortical malformations, resulting in malfunction during postnatal life. Developmental disorders comprise diseases such as Down syndrome, schizophrenia, bipolar disorder, major depressive disorder, autism and epilepsy. Cortical malformations may result from abnormal neuronal proliferation, migration defects or abnormal formation of circuits/synaptogenesis.

In this study, we evaluated whether mice lacking the NF-κB p50 subunit present alterations in cortical structure, with consequent behavioural impairments.

NF-κB p50 subunit-deficient (p50-/-) and wild type mice were analyzed in terms of cortical structure and behavioural abnormalities. All experiments were conducted on male mice.

We found that p50-/- mice at post-natal day 2 (P2) present an increase in radial glial cells, an increase in Reelin protein expression levels, other than a specific alteration in the cortical layering. Moreover, adult p50-/- mice display abnormal columnar organization in the somatosensory cortex, with an increase in cell density, less neuropil space and a loss of linearity in the vertical organization of the minicolumns, a specific decrease in somatostatin- and parvalbumin-expressing interneurons, altered neurite orientation and a concomitant decrease in Synapsin I protein levels. Concerning behaviour, p50-/- mice, other than an increase in locomotor and exploratory activity measured in the open field test, present impairments in social behaviours, scored by means of the three chambered apparatus and the reciprocal social interaction test, with a reduction in social interaction. Finally, we tested the effect of Risperidone, an atypical antipsychotic drug used to treat irritability in autistic patients on p50-/- and wild type mice. Risperidone treatment improved the open field test performance of p50-/- mice, reducing both the distance travelled end the movement speed, decreasing hyperactivity.

Together, these data provide new insight on the possibility of a link between altered function of NF-κB and the pathogenesis of neurodevelopmental disorders. We propose NF-kB p50 subunit-lacking mice as a new mouse model of autism.

P 2/09

FROM NGF SIGNALING TO MIR-219-5P: OLIGODENDROCYTES' DIFFERENTIATION AND MYELINATION

Turturro Sabrina[1], Brandi Rossella[1], Arisi Ivan[1], Scardigli Raffaella[2], Giorgi Corinna[1], Storti Andrea Ennio[3], Ricevuti Flavia[4], La Regina Federico[1], D''Onofrio Mara[1], Cattaneo Antonino[5]

[1]European Brain Research Institute "Rita Levi-Montalcini" ~ Roma ~ Italy, [2]European Brain Research Institute "Rita Levi-Montalcini" - IFT, CNR ~ Roma ~ Italy, [3]Gene Center Regulation, LMU ~ Munich ~ Germany, [4]Mediolanum Farmaceutici ~ Roma ~ Italy, [5]European Brain Research Institute "Rita Levi-Montalcini", Roma, Italy - Scuola Normale Superiore (SNS) ~ Pisa ~ Italy

The intricate signaling between neurotrophins and cytokines governs myelin repair and supports the role of NGF as a key regulator of oligodendrocytes (OL) wellbeing and myelinization. This regulatory homeostatic process could play a significant role in white matter disorders, such as Multiple Sclerosis (MS). The current view is that NGF promotes myelination in the peripheral nervous system, while inhibiting it in the CNS. In agreement with an inhibitory role of NGF on myelination by OLs, we have demonstrated that miR-219-5p, a key positive regulator of oligodendrogenesis, is up-regulated in a mouse model of postnatal NGF neutralization (AD11 Tg mice).
We characterized the interplay between NGF and miR-219-5p in OLs derived from adult neural stem cells (aNSCs), in order to understand the cellular and molecular mechanisms underlying OL differentiation and MBP production. This might provide information for the development of novel therapeutic targets for remyelination.

The specific aims are the following: 1) Understanding how NGF signaling regulates miR-219-5p to promote oligodendrogenesis in WT and AD11-derived OL precursor cells (OPCs); 2) characterizing the role of proNGF/NGF imbalance on miR-219-5p modulation; 3) modulating miR-219-5p expression in order to promote OLs differentiation and myelination. OPC cultures have been established from adult neural stem cells (aNSCs) derived from WT and AD11 Tg mice dentate gyrus in order to: i) characterize the expression of NGF receptors (p75, TrkA, Sort) during the different stages of oligodendrogenesis, by Immunofluorescence and qReal-Time PCR experiments; ii) modulate miR-219-5p expression by Lentiviral delivery of miR-219-5p or of anti-miR-219-5p; iii) modulate miR-219-5p expression by NGF or anti-NGF administration.

Our data shows that AD11 aNSCs express higher levels of miR-219-5p compared to WT and give rise to more O4-positive OL, when differentiated in culture. In addition to that, NGF treatment of AD11 aNSCs down-regulates miR-219-5p expression and inhibits OL differentiation; conversely, anti-NGF administration to WT aNSCs down-regulates miR-219-5p and reproduces the AD11 OLs phenotype.

Our data demonstrates that NGF controls oligodendrogenesis by modulating miR219-5p levels. We are currently characterizing the molecular pathways underlying this modulation, in an attempt to identify new therapeutic targets for remyelination.

P 3/09

MULTIGENERATIONAL EFFECTS OF PRENATAL EXPOSURE TO VALPROIC ACID ON NEURODEVELOPMENT: A PRECLINICAL STUDY

Tartaglione Anna Maria[1], Ricceri Laura[1], Perrone Benedetta[1], De Felice Alessia[1], Balestrieri Emanuela[2], Sinibaldi--Vallebona Paola[2], Calamandrei Gemma[1]

[1]Dept. of Cell Biology and Neuroscience, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Dept. of Experimental Medicine and Surgery, University of Rome Tor Vergata ~ Rome ~ Italy

Clinical evidence indicates that exposure to the antiepileptic agent valproic acid (VPA) during the first trimester of pregnancy increases the risk of congenital malformations and neurodevelopment delay in children; fetal VPA exposure is also associated with a 7-10x increase in relative risk for autism (Ornoy et al. 2015). In rodents, a single VPA administration in pregnancy induces birth defects, deficits in neurodevelopment, and cognitive/social anomalies in the offspring. VPA is a non-selective inhibitor of histone deacetylases of class I and II expressed in the brain. The mechanisms by which VPA causes neurotoxicity are still unknown. Thus rodent models of fetal exposure to VPA represent a tool to study the implication of epigenetic mechanisms in human neurodevelopmental disorders.

The aim of the present study was to evaluate the multigenerational impact of VPA exposure in pregnancy on neurobehavioural development of laboratory mice, by studying both F1 and F2 generation after a single injection of VPA on gestational day (GD) 10.5.

F1: on GD 10.5, pregnant CD1 mice were injected subcutaneously with 500 mg/kg/bw VPA. Offspring of both sexes from each Veh- and VPA-treated litter were assessed for motor and somatic growth, spontaneous locomotion, ultrasonic vocalization, and nest-odour recognition from postnatal day 4 to 12. Motor activity, social abilities and cognitive functions were also analysed at the juvenile and adult stage. F2: At adulthood male and female mice of the F1 generation was mated creating three experimental groups (maternal VPA/paternal vehicle, maternal vehicle/paternal VPA, maternal vehicle/paternal vehicle) to evaluate the contribution of parental exposure to VPA to multigenerational effects on the behavioural phenotype. The same neurobehavioral assessment as in F1 was performed on F2 generation.

In F1, VPA offspring presented tail malformation (“kinky tail”), delay in somatic growth and motor development, hyperactivity and a reduced number of ultrasonic vocalizations. No prenatal treatment-induced differences were evident in adult performance.
In F2, similar alterations in motor development as in F1 were observed in VPA offspring, but with a different profile of effects depending on whether VPA exposure came from the paternal or maternal germline.

Our findings indicate that in utero VPA induces neurodevelopmental alterations that persist also in F2. We are presently analysing molecular markers of VPA effects on early brain development in both F1 and F2 generation, based on previous results indicating endogenous retroviruses (mERV) as possible downstream effectors of the VPA induced epigenetic alteration.

P 4/09

MATERNAL IMMUNE ACTIVATION AFFECTS DOPAMINE NEURON SPONTANEOUS ACTIVITY IN THE OFFSPRING

De Felice Marta[1], Aroni Sonia[1], Muntoni Anna Lisa[2], Frau Roberto[1], Pardu Alessandra[1], Devoto Paola[1], Pistis Marco[1]

[1]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy ~ Cagliari ~ Italy, [2]CNR Neuroscience Institute, Cagliari, Italy ~ Cagliari ~ Italy

Prenatal immune system activation is associated with a later risk to develop neuropsychiatric disorders, like schizophrenia (Boksa, 2010). Hence, immune response factors affect offspring brain maturation, contributing to the emergence of pathological phenotypes, at adulthood. Strong evidence suggests that ventral tegmental area (VTA) dopamine (DA) neurons and their target regions play key roles in the etiology of psychoses. However, it remains unclear whether DA activity and functionality are altered in maternal immune activation models of schizophrenia.

On the bases, we used an immune-mediated neurodevelopmental disruption model. This model mimics a viral infection and recapitulates behavioral and cognitive abnormalities relevant to psychiatric disorders in offspring.

Pregnant rats were injected at gestational day 15 with the proinflammatory cytokine inductor polyriboinosinic-polyribocytidilic acid [poly(I:C), 4 mg/kg i.v] or vehicle. To validate our model, the offspring were behaviorally assessed at adulthood for deficits in sensorimotor gating. Pre-pulse inhibition (PPI) of acoustic startle reflex were examined at PND 60-70. Subsequently, we performed in vivo single cell extracellular recordings in urethane-anesthetized offspring at PND 75-90.

Statistical analyses revealed that male poly(I:C) prenatally-treated offspring displayed abnormal PPI (n=15 and n=23 rats from controls and poly(I:C), Student's t-test, $P<0.05$). Moreover, they showed a lower number and mean frequency of spontaneously active VTA DA neurons when compared to controls (controls vs poly(I:C), n=145 and n=103 cells from n=14 and n=15 rats, respectively; Student's t-test $P<0.01$). In addition, burst parameters of DA cells were strongly altered by poly(I:C) administration (n=125 and n=77 cells from controls and poly(I:C), respectively): mean burst duration and mean spikes per burst were reduced (Student's t-test $P<0.01$), whereas mean intraburst frequency was increased (Student's t-test $P<0.001$). Notably, no differences in any basal electrophysiological properties of VTA DA neurons were detected in female offspring (n=118 cells from 12 rats vs n=122 cells from 17 rats, controls vs poly(I:C), Student's t-test $P>0.05$).

Overall, our findings provide evidence of disrupted activity and functionality of DA neurons following exposure to a prenatal risk factor in male but not in female rats.

P 5/09

REPEATED MATERNAL SEPARATION IN C57BL/6J MICE: CHANGES IN HIPPOCAMPAL GABAERGIC AND GLUERGIC SYNAPTIC FUNCTION AND INCREASED VOLUNTARY ETHANOL CONSUMPTION

Gorule Ashish Avinash[1], Talani Giuseppe[2], Licheri Valentina[1], Biggio Giovanni[1], Sanna Enrico[1]

[1]Department of Life and Environmental Sciences, Section of Neuroscience, Centre of Excellence for the Neurobiology of Dependence, University of Cagliari ~ Monserrato, 09042 Cagliari ~ Italy, [2]Institute of Neuroscience, National Research Council ~ Monserrato, 09042 Cagliari ~ Italy

Exposure to negative and stressful events early in life can lead to a higher risk for neuropsychiatric diseases such as mood and anxiety disorders as well as increased vulnerability to drug abuse in adulthood. The repeated maternal separation (RMS) in rodents is considered an useful model for investigating the long-term consequences of early life stress on brain development and function as well as ethanol (EtOH) sensitivity in adult animals.

In the present study we evaluated the long-term effects of RMS on EtOH voluntary consumption in adult C57BL/6J mice and the possible associated modifications on the function of GABAergic and GLUergic synapses in the hippocampal CA1 field and dentate gyrus (DG).

Pups were separated daily from the dam for 360 min (from PND 3 to 17). On PND 60, mice were tested for their voluntary EtOH consumption by using the two-bottle free choice paradigm. Patch-clamp and field extracellular recordings were performed in hippocampal slices in order to evaluate GABAergic and GLUergic transmission as well as long term synaptic plasticity.

RMS mice showed a significant increased preference as well as intake of EtOH compared to control animals. Patch clamp experiments revealed a significant increase of sIPSC frequency in CA1 pyramidal neurons and a marked enhancement (44.6 ± 10% vs. control) in the modulatory action of THIP on tonic currents in DGGCs. In DGGCs from RMS animals we also observed a marked increase in GLUergic sEPSCs compared to controls. RMS was also accompanied to a decrease in LTP formation and a marked increase in LTD evaluated in CA3-CA1 excitatory synapses. In a subset of mice, tested during the RMS period (PND 5, 10, and 15), we found a marked increase of sIPSC frequency in DGGCs. Interestingly, a single injection of B-ethinylestradiol on PND2 was able to prevent the increase in tonic currents and EtOH intake and preference associated with RMS.

These results demonstrate that RMS is associated with short and long-lasting effects on GABAergic transmission and GLUergic transmission in the hippocampus that, in turn, markedly affect synaptic plasticity and might be correlated with the observed higher EtOH abuse in adulthood.

This work was founded by a grant #CRP-26052 (LR7/2007) from Regione Autonoma della Sardegna.

P 6/09

DIFFERENTIATION AND MIGRATION OF NEURAL STEM CELLS (NSCS) DURING PRECONDITIONING IN A MOUSE MODEL OF NEONATAL HYPOXIA ISCHEMIA

Brancaccio Paola[1], Anzillotti Serenella[2], Cerullo Pierpaolo[1], Cuomo Ornella[1], Di Renzo Gianfranco[1], Annunziato Lucio[1], Pignataro Giuseppe[1]

[1]1Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, "Federico II" University of Naples, [2]2SDN IRCCS, Naples, Italy

Hypoxia-ischemia damage in neonatal brain is a major risk factor of a variety of serious human disorders. The stimulation of endogenous neurogenesis is considered a new potential therapeutic strategy in this disease. Recently, it has been reported that hypoxic and ischemic preconditioning (PC) is able to enhance neurogenesis. PC represents an endogenous neuroprotective strategy in which a subliminal stimulus is able to protect the brain from an harmful stimulus.

The aim of this research project is to investigate whether hypoxic preconditioning is able to stimulate the differentiation and the migration of neural stem cells in a mouse model of neonatal hypoxia ischemia.

Seven-day-old C57BL/6 (P7) mice were divided into different experimental groups. Hypoxia ischemia was induced by using Rice-Vannucci model. Briefly, were subjected to ligation and cutting of the right common carotid artery followed by different time intervals of hypoxia (92% N2 and 8% O2), and reperfusion. Histopathological damage in the hippocampus was determined by measuring the expression level of propidium iodide (PI), whereas the neurogenesis was evaluated by immunohistochemistry with different markers. In addition, developmental sensorimotor reflexes were evaluated by behavioral tests.

As expected, the greatest damage was found in mice subjected to HI 60'and sacrificed 7 days after ischemia induction. Furthermore, a significant reduction in the hippocampal damage was observed in mice subjected to hypoxia 20'(P7) followed, 3 days later, by HI 60'(P10) and sacrificed at P11. Indeed, this result suggests that 20' hypoxia functions as a preconditioning stimulus. The damage was mainly localized in CA1 and CA3 region, whereas the dentate gyrus was spared. Concerning the dentate gyrus we found that preconditioning stimulus was able to trigger an increased expression of nestin, a marker of neurogenesis. In addition, preconditioned animals showed a better performance in the geotaxis reflex test.

Our results indicate that preconditioning is associated to neurogenesis also in immature brain and pave the rood for further studies aimed at analyzing the molecular basis of neurogenesis.

P 7/09

THE CHRNA7 AND ITS DUPLICATED ISOFORM, CHRFAM7A, GENE REGULATION AND EXPRESSION IN NEURONAL AND IMMUNE CELL MODEL

Maroli Annalisa[1], Fornasari Diego[1], Benfante Roberta[2]

[1]Dept. of Medical Biotechnology and Translational Medicine (BIOMETRA), University of Milan, Via Vanvitelli 32, 20129 – Milan (Italy), +390250316945 ~ Milano ~ Italy, [2]CNR –Neuroscience Institute, via Vanvitelli 32, 20129 – Milan (Italy) ~ Milano ~ Italy

The alpha7 neuronal nicotinic receptor, CHRNA7 (Chr. 15, 15q13-q14 region) , is a homopentameric ligand gated ion channels with high permeability to Ca2+. It is widely expressed in both the brain, where presynaptically it modulates the release of neurotransmitters and postsynaptically, its activation lead to changes in gene expression, and periphery with multiple important role in cognition and the immune system. In the periphery, CHRNA7 is expressed in neuroendocrine cells of the lung, in keratinocytes, bone marrow, sperm and macrophages. Here, it has a role of modulator of the inflammatory response through the “cholinergic anti-inflammatory pathway”, whose activation prevents the release of cytokines such as TNFalpha, IL-6, IL-8 and HMGB1. Decreased expression and function of CHRNA7 have been associated with many diseases including schizophrenia, bipolar disorder, ADHD, Alzheimer’s disease, autism, epilepsy, RETT syndrome and learning disorders. Recently, CHRFAM7A gene was discovered. It is the product of a recombination event that occurred in human where exon 5 to 10 of CHRNA7 gene fused to four novel exons A, B and C (FAM7A gene) from the serine/threonine kinase ULK4 gene, and exon D of unknown provenance, located 1.6 Mb apart from CHRNA7 gene, in the direction of the centromere, and in the opposite orientation with respect to CHRNA7. In vitro experiments have shown that CHRFAM7A protein assembles with alpha7 resulting in a dominant negative regulation of its function. Thus, the number of copies of CHRFAM7A can regulate CHRNA7 function.

The promoter for the CHRFAM7A gene has not been characterized so far: in the present study we give new insights on the molecular mechanisms contributing to the CHRFAM7A gene expression.

For this purpose, we use two different models, a human monocyte cell line, THP-1, and a neuroblastoma cell line, SH-SY5Y.

The gene encoding CHRFAM7A is expressed both in cells of innate immunity and in neuronal cells; experiments in the two cell models revealed the presence of different regulatory tissue-specific regions.

These regulatory regions are important for proper CHRFAM7A gene expression in different tissues.

P 8/09

NURR1 AND LMX1A TRANSCRIPTION FACTORS COOPERATE TO PROMOTE DOPAMINERGIC DIFFERENTIATION

Volpicelli Floriana[1], Pulcrano Salvatore[2], De Sanctis Claudia[2], Perrone Capano Carla[1], Di Porzio Umberto[2], Bellenchi Gian Carlo[2]

[1]University of Naples "Federico II", Department of Pharmacy ~ Naples ~ Italy, [2]Institute of Genetics and Biophysics ''Adriano Buzzati Traverso'', CNR ~ Naples ~ Italy

Mesencephalic dopaminergic (mDA) neurons control voluntary movement and emotions. Understanding their development will help to unveil the pathophysiology of Parkinson's disease and other neuropsychiatric disorders and to conceive new therapeutical approaches. Specific temporal and environmental cues are required for mDA development and maintenance. Among these, the transcription factors Lmx1a and Nurr1 are important mediators.

To elucidate whether Nurr1 and Lmx1a can act in a cooperative manner to promote mDA differentiation.

Molecular Cloning and Viral Infection: E11 ventral midbrain cells, plated into a media containing mitogens at a density of 50.000 cells/cm2, are infected at 3DIV with Nurr1 and Lmx1a cDNAs, cloned into lentiviral vectors under the control of the tetracycline operator. At 6DIV the cells are differentiated in absence of mitogens and in presence of ascorbic acid and collected at 12DIV.
Real time PCR: Total RNA, isolated using Tri-Reagent, is reverse transcribed. The gene expression levels are quantified using 7900HT Fast Real-Time PCR System and normalized to those of the housekeeping gene HPRT. Western blot analysis: 50 µg/lane of total proteins are separated on 10% SDS polyacrylamide gel and transferred to PVDF membrane. After blocking, the membranes are incubated for 2hr at RT with the primary antibody and subsequently with HRP-secondary antibody (1:10000). The reactions are detected with ECL system.
Immunocytochemistry: Cell cultures fixed in 4% paraformaldehyde are permeabilized for 15 min in 0.1% Triton X-100 and 10% normal goat serum and incubated for 2 hr at RT or overnight at 4°C in the primary antibodies solution. Transient transfection and luciferase assay: NBRE sequence, cloned into a pGL3 basic vector, is co-transfected with the 3x-FLAG-Nurr1 or V5-His-Lmx1a plasmid into HeLa cells using lipofectamine 2000. Renilla luciferase vector is used as an internal control.

Real time PCR, western blot and immunofluorescence analyses show that Nurr1 and Lmx1a co-expression decreases the expression of the glial marker GFAP, whilst GABAergic or serotonergic phenotypes are not affected. Concomitantly, Nurr1 and Lmx1a co-expression leads to an enhanced mDA phenotype, increasing the expression of mDA genes (TH, VMAT2 and DAT) and the number of TH+ cells. Interestingly, luciferase reporter assays using Lmx1a and the truncated constructs of Nurr1 demonstrate that the C-terminal portion of Nurr1 is necessary for the synergistic activity.

P 9/09

THE SEROTONIN RECEPTOR 7 STIMULATES NEURITE ELONGATION THROUGH CDK5, MTOR AND CDC42 PATHWAYS AND MODULATES DENDRITIC SPINES MORPHOLOGY

Speranza Luisa[1], Molinari Marta[2], Volpicelli Floriana[3], De Stefano Maria Egle[4], Lombardi Loredana[4], Bellenchi Gian Carlo[1], Di Porzio Umberto[1], Crispino Marianna[2], Perrone--Capano Carla[3]

[1]Institute of Genetics and Biophysics "Adriano Buzzati Traverso", CNR ~ Naples ~ Italy, [2]Department of Biology, University of Naples Federico II ~ Naples ~ Italy, [3]Department of Pharmacy, University of Naples Federico II ~ Naples ~ Italy, [4]Department of Biology and Biotechnology "Charles Darwin", Istituto Pasteur Fondazione Cenci Bolognetti, University of Rome La Sapienza ~ Rome ~ Italy

The serotonin receptor 7 (5-HT7R) influences neuronal morphology during embryonic stage, regulates synaptic plasticity during early postnatal development and is involved in diverse physio-pathological processes of the CNS (Kobe et al., 2012; Gellynck et al., 2013; Speranza et al., 2013).

We investigate the role of the 5-HT7R in the remodeling of neuronal cytoarchitecture and connectivity in telencephalic CNS circuits. In particular, we study the cellular and molecular mechanisms of the neurite outgrowth induced by the stimulation of 5-HT7R and the associated signaling transduction pathways. The structural plastic changes in the mature brain are studied after pharmacological stimulation of the receptor in adult mice.

Primary cultures from several embryonic areas of murine CNS (cortex, hippocampus, striatal complex) are pharmacologically stimulated by selective agonists (LP-211) and antagonists of the 5-HT7R, with or without inhibitors of key molecules involved intracellular pathways. The effects on neuronal morphology and signal transduction pathways are analyzed by immunofluorescence, RT-PCR, WB. For morphological analyses, fixed cells are immunostained against neuron specific class III β-tubulin and images processed using the Image J software.
Axonal elongation is analysed on hippocampal neurons plated in microfluidic chambers, and maintained in vitro for 6 days with or without selective 5-HT7R agonists.
For in vivo analyses, one month-old mice are injected with LP-211 for three days. Analyses of dendritic spine morphology are performed on z-stack images from DiI-labeled brain slices.
We show that pharmacological stimulation of 5-HT7R enhances neurite outgrowth in embryonic neuronal primary cultures, through multiple signal transduction pathways (ERK, mTOR, the Rho GTPase Cdc42, Cdk5). All these signaling systems are known to converge on the reorganization of cytoskeletal proteins that subserve neurite outgrowth. Indeed, selected cytoskeletal proteins are affected by pharmacological stimulation of the receptor, and drugs modulating actin dynamics interfere with 5-HT7R-induced neurite elongation. Using microfluidic chambers we demonstrate that activation of 5-HT7R stimulates axonal elongation. Preliminary results suggest that stimulation of 5-HTR7 in vivo increases density, length and diameter of the dendritic spines in the dorso-lateral striatum.

Our results identify for the first time several signal transduction pathways, activated by stimulation of 5-HT7R, that converge to promote cytoskeleton reorganization and consequent modulation of axonal elongation during development. In the adult brain, the activation of the receptor modulates morphology and number of dendritic spines. Therefore, the activation of 5-HT7R might represent one of the key elements regulating CNS connectivity and plasticity.

P 10/09

INVOLVEMENT OF THE NA+/CA2+ EXCHANGER ISOFORM 1 (NCX1) IN NGF-INDUCED NEURONAL DIFFERENTIATION THROUGH CA2+-DEPENDENT AKT PHOSPHORYLATION

Secondo Agnese, Esposito Alba, Sirabella Rossana, Boscia Francesca, Pannaccione Anna, Molinaro Pasquale, Cantile Maria, Ciccone Roselia, Sisalli Maria Josè, Scorziello Antonella, Di Renzo Gianfranco, Annunziato Lucio

Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine,"Federico II" University of Naples ~ Napoli ~ Italy

Nerve growth factor (NGF) induces neuronal differentiation by modulating intracellular Ca2+ concentrations ([Ca2+]i). However the role of the main Ca2+-extruding system Na+/Ca2+ exchanger (NCX) in neuronal differentiation remains unexplored.

In this study we investigated whether the three isoforms of NCX could participate to neuronal differentiation by modulating Ca2+ content in endoplasmic reticulum (ER) and Akt pathway.

Neuronal differentiation was studied in PC12 cells exposed to NGF and in rat primary cortical neurons at different DIV. NCX1 was upregulated or downregulated by transfecting its neuronal splicing isoform NCX1.4 or specific Duplexes against this isoform with Lipofectamine 2000. NCXs, GAP-43, MAP2, and phospho-Akt protein expression was evaluated by Western blot, immunocytochemistry and confocal microscopy. NCX currents (INCX) and [Ca2+]i were monitored by means of patch-clamp in whole-cell configuration and Fura-2AM single-cell video imaging, respectively.

NGF caused progressive neurite elongation with a significant increase of the well known marker of growth cones, GAP-43. Furthermore, an enhancement of ER Ca2+ content and of Akt phosphorylation through an early activation of ERK1/2 was also detected. During NGF-induced differentiation, while the NCX1 protein level increased, NCX3 decreased, and NCX2 remained unaffected, total INCX increased. Moreover, NCX1 colocalized and coimmunoprecipitated with GAP-43, and NCX1 silencing prevented NGF-induced effects on GAP-43 expression, Akt phosphorylation, and neurite outgrowth. On the other hand, the overexpression of NCX1.4, even in the absence of NGF, induced an increase in Akt phosphorylation and GAP-43 protein expression. Interestingly, tetrodotoxin-sensitive Na+ currents and SBFI-detected [Na+]i significantly increased in cells overexpressing NCX1.4 as well as ER Ca2+ content. This latter effect was prevented by tetrodotoxin. Furthermore, either the [Ca2+]i chelator BAPTA-AM or the PI3K inhibitor LY 294002 prevented Akt phosphorylation and GAP-43 protein expression rise in NCX1.4 overexpressing cells. Moreover, in primary cortical neurons, NCX1 silencing prevented Akt phosphorylation, GAP-43 and MAP2 overexpression, and neurite elongation.

Overall our results suggest that NCX1 participates to neuronal differentiation through the modulation of ER Ca2+ content and PI3-K signaling.

P 11/09

INFLAMMATORY RELATED PATHWAYS AND SGK1 SIGNALING AS TARGETS OF EARLY LIFE STRESSFUL EVENTS: ROLE OF DNA METHYLATION AND MIRNAS

Plazzotta Giona[1], Luoni Alessia[2], Riva Marco Andrea[2], Pariante Carmine Maria[3], Cattaneo Annamaria[3]

[1]IRCCS Fatebenefratelli ~ Brescia ~ Italy, [2]Department of Pharmacological and Biomolecular Sciences, University of Milan ~ Milan ~ Italy, [3]Stress, Psychiatry and Immunology Lab & Perinatal Psychiatry Department of Psychological Medicine, Institute of Psychiatry, Department of Psychological Medicine, King's College London ~ London ~ United Kingdom

Stress and glucocorticoid hormones regulate hippocampal neurogenesis, but the molecular mechanisms mediating these effects are poorly understood. Using human neural stem cells, we have previously shown that cortisol increases SGK-1 expression that in its turn regulates neurogenesis activity and Glucocorticoid Receptor (GR) function.

Here we aimed at characterizing the impact of early life stressful events on SGK-1 signaling pathway and the role of epigenetic mechanisms in SGK1 modulation.

Using a cross species approach we first analyzed mRNA expression levels of SGK1 (RT-PCR) in the hippocampus of male and female adult rats (PND62) exposed or not to a prenatal stress (PS) paradigm and we also evaluated SGK1 modulation in the human blood cells (whole blood mRNA using PaxGene Tubes) of both controls and depressed patients characterized for childhood trauma experiences. In order to investigate the long-term effect on SGK1 alterations, we also investigated epigenetic mechanisms such as DNA methylation and miRNAs.

We found a significant increase in mRNA levels of SGK1 both in male and female exposed animals (+48%, $p < 0.005$ PS vs CTRL in males; +24%, $p < 0.005$ PS vs CTRL in females). Interestingly, we also found that SGK1 mRNA levels were significantly increased in the group of subjects with a history of trauma as compared to those who have not experienced trauma (mean of the relative expression ratio: 1.3 ± 0.4 in the subjects with no trauma and 2.6 ± 0.8 in subjects with trauma, +100%, $p = 0.02$; CTRL, $p<0.05$). Depressed patients showed higher SGK1 levels (+30% vs. controls, $p < 0.05$) as compared to controls. Most importantly, the SGK1 increase was higher in those who had both childhood trauma and depression (+45% $p < 0.01$ vs. controls), with significant statistical interactions between groups ($p<0.05$). Furthermore, we observed a common hyper methylation within SGK1 gene in both the species and a down-regulation of a panel of miRNAs that target SGK1 including miR-204-3p, rno-miR-151-3p and mir-711 both in the hippocampus of PNS animals and in the blood of subjects exposed to childhood trauma.

Our data indicate that an exposure to early life stressful event cause activation of pathways involved in inflammation and in SGK1 signaling which are associated with enhanced vulnerability for depression development. Moreover the persistence of changes over time is associated with changes in a panel of miRNAs rather than changes in DNA methylation.

P 12/09

INVOLVEMENT OF MUSCLE-SPECIFIC MIRNA-206 IN THE SPINAL MUSCULAR ATROPHY PATHOGENESIS

Boido Marina, Valsecchi Valeria, De Amicis Elena, Piras Antonio, Vercelli Alessandro

Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience ~ Turin ~ Italy

Spinal muscular atrophy (SMA) is a fatal paediatric genetic disease, characterized by motor neuron (MN) death, leading to progressive muscle weakness, respiratory failure, and, in the most severe cases, to death. SMA is due to the deletion or mutation of the telomeric survival MN gene (SMN1), on chromosome 5. Its homologous, SMN2 gene, only produces a limited amount of functional protein which can modulate SMA severity. Specific or general changes in the activity of ribonucleoprotein containing micro RNAs (miRNAs) play a role in the development of SMA. Additionally miRNA-206 has been shown to slow amyotrophic lateral sclerosis (ALS) progression by promoting a compensatory regeneration of neuromuscular synapses.

Seen the tight relationship between SMN and miRNPs complexes, and the neuroprotective role of miRNA-206 in the regeneration of the neuromuscular junction (NMJ) in ALS (a neurodegenerative disease with different etiology, characterized, similarly to SMA, by loss of motor neurons, denervation, atrophy and paralysis of target muscles), we investigated the miRNA-206 pathway in a mouse model of intermediate SMA (SMAII), the SMNdelta7 mice.

We correlated the morphology and the architecture of NMJs of the quadriceps, a muscle which is affected early in SMA, with the expression levels of miRNA-206 in a murine model of SMA.

Our results showed a decrease in the percentage of type II fibers, an increase in atrophic muscle fibers and a remarkable accumulation of neurofilament (NF) in the pre-synaptic terminal of the NMJs in the quadriceps of SMA II mice. Furthermore, molecular analysis highlighted a direct link between miRNA-206-HDAC4-FGFBP1, and in particular, a strong up-regulation of this pathway in the late phase of the disease.

We propose that miRNA-206 is activated as survival endogenous mechanism, although not sufficient to rescue the integrity of motor neurons. We speculate that early modulation of miRNA-206 expression might delay SMA neurodegenerative pathway and that miRNA-206 could be an innovative, still relatively unexplored, therapeutic target for SMA.

Supported by Girotondo ONLUS, Smarathon ONLUS and University of Turin grants.

P 13/09

ORIGIN AND GENERATION OF DIFFERENT ASTROGLIAL PHENOTYPES IN THE CEREBELLUM

Cerrato Valentina[1], Parmigiani Elena[1], Leto Ketty[1], Fucà Elisa[1], Figueres--Oñate María[2], López Mascaraque Laura[2], Buffo Annalisa[1]

[1]NICO (Neuroscience Institute Cavalieri Ottolenghi), Dept of Neuroscience Rita Levi Montalcini, University of Turin ~ Turin ~ Italy, [2]Cajal Institute ~ Madrid ~ Spain

The anatomical and functional complexity of the cerebellum is reflected by a remarkable heterogeneity of neuronal and astroglial subsets, with highly distinctive morphological and spatial features. While mechanisms of neuronal diversification have been partly clarified, astrogliogenesis remains poorly explored.

Here we addressed the origin(s) and the genesis of the repertoire of astroglial types.

Firstly, in order to study the lineage relationships among different astroglial phenotypes, we performed in vivo clonal analysis using Star Track plasmids and Confetti mice. Furthermore, the proliferative behavior of astrocyte precursors in distinct cerebellar layers was investigated through double-thymidine analogues and birthdating analysis. The role of environmental instructive cues was then explored by means of homo/heterochronic transplants.

In vivo clonal analyses revealed clones containing both cortical protoplasmic and white matter (WM) fibrous astrocytes. Clone composition indicates the existence of three major embryonic progenitor types producing either granular layer astroglia, or WM astrocytes, or a mixed progeny composed of Bergmann glia (BG) and granular layer (GL) astrocytes. Postnatally, these latter clones are likely to derive from radial progenitors located in the PCL dividing in situ to generate both BG and GL astroglia. Furthermore, WM astroglial clones appear smaller compared to those in the cortical layers. Double-thymidine analogues and birthdating analysis showed that these differences in clone size were associated with distinct proliferative behavior of astrocyte precursors in distinct cerebellar layers. In particular, those of the PCL (i.e. immature BG) revealed the highest division rate. Additionally, they displayed different proliferative rhythms in crowns and fissures during early postnatal development, suggesting a contribution to cerebellar foliation. Eventually, homo and heterochronic transplants revealed that specific astroglial phenotypes are instructed by temporally defined extrinsic signals.

In conclusion, our study reveals that cerebellar astrogliogenesis occurs from distinct embryonic progenitors according to a well-defined spatiotemporal pattern.

P 14/09

ACTION OF MEMANTINE ON STRIATAL SYNAPTIC PLASTICITY

Mancini Maria [1], Ghiglieri Veronica[2], Bagetta Vincenza[1], Pendolino Valentina[1], Vannelli Anna[1], Cacace Fabrizio[1], Mineo Desirée[1], Calabresi Paolo[2], Picconi Barbara[1]

[1]IRCCS Fondazione Santa Lucia ~ Roma ~ Italy, [2]Università degli Studi di Perugia ~ Perugia ~ Italy

Memantine is an open channel blocker that antagonizes NMDA-receptors reducing the inappropriate Ca2+ influx occurring in the presence of moderately increased glutamate levels but at the same time it preserves the ability of the transient physiological activation by higher glutamate concentration essential for learning and memory formation at synaptic level.

The aim of the present study was to investigate whether the treatment with memantine could affect the striatal synaptic plasticity and interfere with the long-term potentiation (LTP) process.

The effects of memantine were studied in vitro on coronal cerebral slices. Excitatory postsynaptic potentials (EPSPs) were recorded in MSNs in the dorsolateral striatum by intracellular sharp electrodes and whole cell patch clamp experiments before and after the HFS (high frequency stimulation) protocol induction.

The experiments performed revealed that the exposure to memantine elicited a disruption of the LTP induction and maintenance and revealed, in the majority of the recorded neurons, a LTD, which was concentration dependent (0.3-10 µM). Interestingly, the preincubation with the D2 DA receptor antagonist sulpiride (10 µM) prevented memantine-induced LTD and restored LTP. Moreover, the D2 DA agonist quinpirole (10 µM), similarly to memantine, induced a LTD in a subgroup of MSNs. In addition, memantine-induced LTD was also prevented by the CB1 endocannabinoid receptor antagonist AM251 (1 µM) which inhibits the endocannabinoid retrograde signal at striatal synapses.

These results suggested that the actions exerted by memantine on striatal synaptic plasticity, and in particular the LTD observed in MSNs, could be attributed to its ability to interact with a signaling system related to D2 DA receptors. By contrast, the blockade of NMDA receptor seemed not to be involved in memantine-induced LTD since APV (30 µM) and MK801 (10 µM), two NMDA receptor blockers, failed to induce this form of synaptic plasticity. As a whole, our data indicate that memantine could find a new application for the treatment of neurological disorders where D2 DA receptor represents a possible therapeutic target and more caution should be actually used in the current therapies in which memantine is used.

P 15/09

A NOVEL CAMP/PKA/APP/ASS PATHWAY MODULATES HIPPOCAMPAL LTP

Rebosio Claudia[1], Rivera Daniela[2], Pronzato Maria Adelaide[2], Puzzo Daniela[3], Arancio Ottavio[4], Ricciarelli Roberta[2], Fedele Ernesto[1]

[1]Department of Pharmacy, Section of Pharmacology and Toxicology, University of Genoa ~ Genoa ~ Italy, [2]Department of Experimental Medicine, Section of General Pathology, University of Genoa ~ Genoa ~ Italy, [3]Department of Bio-Medical Sciences, Section of Physiology, University of Catania ~ Catania ~ Italy, [4]Department of Pathology and Cell Biology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University ~ New York ~ United States

Pharmacological and genetic manipulations of the cAMP/PKA/CREB pathway influence hippocampal late LTP and long-term memory. Specifically, inhibition of PDE4-mediated cAMP breakdown enhances LTP and improves memory.
Recent studies indicate that picomolar concentrations of amyloid beta (Aβ), normally produced in the brain, exert physiological functions. Indeed, Aβ potentiates hippocampal LTP and improves memory, while its depletion does the opposite.

Since LTP is dependent on both cAMP and Aβ, we investigated the neurochemical relationships between these two signalling molecules.

APP and Aβ have been measured respectively by immunoblot and ELISA in mouse N2a cells, stably expressing WT human APP695, and rat hippocampal slices following different drug treatments. Immunoprecipitation and RNA interference experiments have been performed in N2a cells. Hippocampal LTP was recorded in the CA1 region following Schaffer collateral pathway stimulation with a weak tetanus in slices from WT or APP KO mice

Our results show that rolipram (ROL 0.1–10 µM), 8Br-cAMP (1 µM-1 mM) or forskolin (FSK 1µM-10µM) increased APP and Aβ levels in mouse N2a cells. Similar results were obtained with the PKA activator 6-MB-cAMP (1-100 µM) but not with the EPAC activator 8-pCPT-2-O-Me-cAMP-AM (0.01-25 µM). In addition, the FSK (1 µM)- and the 6-MB-cAMP (100 µM)-induced effects were blocked by cycloheximide (80 µg/ml) but not by actinomycin D (4 mg/ml). The effects of ROL (100 µM), FSK (100 µM) or 6-MB-cAMP (100 µM) on APP and Aβ levels were reproduced also in rat hippocampal slices.
To identify post-transcriptional mechanisms involved in the cAMP-mediated regulation of APP/Aβ production, we analysed whether hnRNP-C and FMRP, two RNA binding proteins regulating APP expression, could be involved. FSK-induced cAMP accumulation was not associated with an increase of APP mRNA in the hnRNP-C immunocomplex, and hnRNP-C or FMRP knocking down did not alter the APP/Aβ response to FSK.
Finally, in WT mice, a weak tetanus elicited LTP in CA1 hippocampal neurons that was potentiated by ROL (100 nM). On the contrary, ROL was devoid of any effect in slices from APP KO mice or in slices from WT animals in the presence of anti-Aβ antibodies (JRF/rAb2, 4 µg/ml; M3.2, 4 µg/ml).

cAMP controls APP and Aβ production through a PKA-dependent mechanism that requires protein synthesis but not gene expression. Neither hnRNP-C nor FMRP seem to mediate the effects of

cAMP.
Our data demonstrate the existence of a novel cAMP/PKA/APP/Aβ pathway that plays a key role in the expression of LTP.

P 16/09

DIFFERENT MOUSE GENETIC MODELS OF HUMAN DYT1 DYSTONIA SHARE CHANGES OF PHOSPHODIESTERASE-10A IN BASAL GANGLIA CIRCUITS

Saverioni Ilaria[1], Cardarelli Silvia[1], D''Angelo Vincenza[2], Castelli Valentina[1], Giorgi Mauro[1], Biagioni Stefano[1], Palumbo Francesca[2], Martorana Alessandro[2], Bonsi Paola[3], Pisani Antonio[2], Sancesario Giuseppe[2]

[1]Department of Biology and Biotechnology, "La Sapienza" University of Rome ~ Rome ~ Italy, [2]Department of Systems Medicine, University of Rome Tor Vergata ~ Rome ~ Italy, [3]Santa Lucia Foundation ~ Rome ~ Italy

DYT1 dystonia is a movement disorder caused by a 3-bp deletion (ΔGAG) in the gene that encodes TorsinA. In most forms of dystonia the brain regions primary affected are thought to have functional rather than structural abnormalities. PDE10A, a key enzyme in the catabolism of cyclic nucleotides, has unique distribution in the basal ganglia. In fact, its mRNA is expressed only in striatal Medium Spiny Neurons and the mature protein is carried to the output nuclei: entopeduncular nucleus and external globus pallidus, involved in direct and indirect pathway, respectively.

We studied whether different mouse genetic models of human DYT1 Dystonia share common changes of Phosphodiesterase-10A (PDE10A) in basal ganglia pathways. PDE10A was first studied in control mice and in mice carrying either human wild-type torsinA (hWT) or mutant torsinA (hMT). The same analyses were performed in a knock-in DYT1 model (Tor1a+/+ and Tor1a+/Δgag mice)

Immunohistochemical analysis was used for identification of PDE10A positive neurons and nerve fibers. Quantitative analysis of PDE10A expression was also assessed by western blotting, using a specific antibody. PDE10A dependent cAMP hydrolyzing activity was evaluated using [3H]-cAMP as substrate and papaverine, a well-known PDE10A-inhibitor, on tissue homogenates of the same nuclei.

Our studies demonstrate that PDE10A expression and activity were clearly increased in the globus pallidus of hMT mice compared to control mice, while in the entopeduncular nucleus PDE10A expression and activity were significantly decreased both in hWT and hMT mice. However, expression of PDE10A mRNA was comparable in the three groups of animals. In the knock-in mouse model we found a decreasing trend in expression and activity of PDE10A in the entopeduncular nucleus respect to wild type. On the other hand, a significant increase in both expression and activity occurs in the globus pallidus of Tor1a+/Δgag animals.

Our findings suggest that genetic changes of torsinA affect expression and/or axonal transport of PDE10A in basal ganglia circuits of the direct and indirect pathways in an opposite way. ΔE-TorsinA could interfere with PDE10A trafficking by mechanisms that are still to be clarified.

P 17/09

IH SUPPRESSION INCREASES SYNAPTIC EXCITABILITY OF MIDBRAIN DOPAMINERGIC NEURONS IN VITRO AND CAUSES SELECTIVE NIGROSTRIATAL DEGENERATION IN VIVO

Carbone Carmen, Masi Alessio, Narducci Roberto, Resta Francesco, Costa Alessia, Provensi Gustavo, Mannaioni Guido

NEUROFARBA ~ FIRENZE ~ Italy

Parkinson's disease (PD), the second most common neurodegenerative disorder worldwide, results from a progressive and selective degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). A still unclear, major histopathological aspect of PD concerns the severe neurodegeneration of SNc DA neurons in comparison to the closely related DA neurons in the neighboring Ventral Tegmental Area (VTA), despite the many similarities in terms of metabolic, physiological, and neurochemical properties. Recently, we demonstrated that acute MPP+, a toxin able to produce preferential SNc DA degeneration, alters the electrophysiological properties of SNc DA neurons in vitro by inhibiting the Hyperpolarization-activated current (Ih

The aim of this work is to define the molecular and physiological mechanism of differential vulnerability between DA neurons in SNc and VTA.

Whole-cell recordings were performed in acute midbrain slices from juvenile WH rats or TH-GFP mice. Simultaneous determination of changes in cytosolic calcium concentration was achieved by loading the recorded neuron with Fluo-4 or Oregon Green. Fluorescence was elicited with a blue LED and detected with a photomultiplier tube. Inactivation of Ih in vivo was obtained by stereotaxic intranigral injection of ZD7288 or ivabradine in adult WH rats or TH-GFP mice.

The results obtained indicate that the pharmacological suppression of Ih increases the amplitude and duration of evoked Excitatory Post-Synaptic Potentials (EPSP) in DA neurons from TH-GFP mice. In particular, Ih suppression leads to temporal summation of multiple EPSPs, indicating reduced ability to resolve single excitatory inputs at somatic level. Moreover, differential response underlines selective vulnerability in SNc compared to VTA DA neurons and depends on postsynaptic Ih magnitude. Also we have obtained preliminary experimental evidence in vivo through intranigral injection of Ih blockers recapitulates the DA degeneration pattern seen in MPP+ PD model.

These findings support the hypothesis that Ih loss of function, possibly caused by PD-trigger mechanisms such as mitochondrial failure and oxidative stress, may act in concert with SNc-specific synaptic connectivity to promote selective vulnerability.

P 18/09

LOCALLY SYNTHESIZED 17BETA-ESTRADIOL MEDIATES SYNAPTIC LONG-TERM POTENTIATION VIA D1-LIKE DOPAMINE SIGNALING IN THE DORSAL STRIATUM

Tozzi Alessandro[1], De Iure Antonio[2], Tantucci Michela[2], Durante Valentina[2], Quiroga--Varela Ana[2], Di Mauro Michela[2], Mazzocchetti Petra[1], Grassi Silvarosa[2], Pettorossi Vito Enrico[2], Calabresi Paolo[2]

[1]Fondazione Santa Lucia IRCCS ~ Roma ~ Italy, [2]University of Perugia ~ Perugia ~ Italy

Estrogens, in particular 17beta-estradiol (E2), play a fundamental role in regulating brain activity modulating synaptic plasticity and influencing cognition and behavior. E2 effects depend on genomic responses via nuclear receptors and via rapid non-genomic responses involving membrane receptors. ERs may be also activated by dopamine (DA) and the D1-like receptor (D1R) agonist SKF-82958, stimulating ER-dependent activation of intracellular signaling pathways. Moreover, in the nucleus striatum E2 activates membrane-localized ERs to modulate DA synaptic neurotransmission, calcium channel activity and motor activity. Therefore, the interplay between DA and E2 is likely to occur for modulating neuronal activity and plasticity in striatal neurons. However, it is unknown whether DA interacts with the circulating E2 or with the E2 that can be locally synthesized in the nervous system from testosterone through P450-aromatase (ARO).

We hypothesized a role for endogenous E2 in striatal synaptic plasticity. Since DA is critical for synaptic plasticity in this structure, we analyzed a possible interaction between E2 and DA in this event.

We performed electrophysiological sharp electrode and patch-clamp recordings of striatal medium spiny neurons (MSNs) and cholinergic interneurons (ChIs) in slice preparations of male rats. Long-term changes of synaptic responses were induced by electrical stimulation of striatal glutamatergic fibers in neurons from control slices and from slices treated with the inhibitor of E2 synthesis letrozole or the ERs antagonist ICI-182780. Compounds able to modulate the D1R and ER signaling pathways were bath-applied to the slice or intracellularly via the patch pipette.

Inhibition of E2 synthesis or antagonism of ERs prevented the long-term potentiation (LTP) induction in MSNs and ChIs. Activation of the D1-like DA receptor/cAMP/PKA-dependent pathway restored LTP. Exogenous E2 reversed the effect of ARO inhibition in MSNs. Also, antagonism of M1Rs prevented the D1-like receptor-mediated restoration of LTP, confirming a role for ChIs in controlling the E2-mediated LTP of MSNs.

Our findings show that LTP in MSNs depends on the presence of DA and on the locally synthesized E2 and suggest that DA can activate ERs by a close interaction between D1Rs and ERs. This co-activation is possibly required to drive intracellular signals able to trigger LTP. We suggest that ER stimulation represents a pivotal step for the long-term modulation of synaptic changes in the striatum. The role of neurosteroids in modulating synaptic plasticity in pathological conditions may be of particular interest, especially for those pathologies involving the nucleus striatum such as Parkinson's disease.

P 19/09

FENOFIBRATE, A CLINICALLY USED PPARA AGONIST, ENHANCES DOPAMINE AND SEROTONIN NEURONAL ACTIVITY

Aroni Sonia[1], De Felice Marta[1], De Montis Maria Graziella[2], Melis Miriam[1], Pistis Marco[1]

[1]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy ~ Cagliari ~ Italy, [2]Department of Molecular and Developmental Medicine, University of Siena, 53100 Siena, Italy ~ Siena ~ Italy

Peroxisome proliferator-activated receptors-alpha (PPARα) is one of three subtypes of the nuclear receptor PPAR family. They are widely expressed in the CNS, and are activated by endogenous ligands, such as fatty acids and eicosanoid derivatives, and by synthetic ligands such as hypolipidemic fibrates.
Preclinical studies demonstrated that activation of PPARα acutely modulates activity of midbrain dopamine neurons via non-transcriptional phosphorylation of β2 subunit-containing nicotinic acetylcholine receptors (β2*nAChR). Experiments also suggest that these effects might underlie antidepressant-like properties, as shown by preliminary behavioral experiments in animal models of depression. Little is known, however, about the actions of chronic treatments with PPARα agonists on neuronal activity, specifically of dopamine and serotonin cells, which are key in affective regulation.

On these bases, we investigated how a chronic administration of fenofibrate, a PPARα agonist clinically available for lipid disorder metabolism, affects dopamine neurons in the ventral tegmental area (VTA) and serotonin cells in the dorsal raphe nucleus (DRN), two brain regions involved in the neurobiology of psychiatric disorders, including depressive states.

In vivo extracellular single unit recordings were carried out from adult anaesthetized rats, which were fed for 14 days with either a standard (control group) or fenofibrate 0.2% (FBR group).

Statistical analysis did not reveal differences in neither the number of spontaneously active VTA dopamine neurons nor their firing rate between control and FBR groups. However, chronic fenofibrate administration enhanced coefficient of variation and burst activity of dopamine cells (i.e. percent of spikes in burst, mean spikes per burst, burst rate, mean intraburst frequency and mean burst duration), which might correlate with higher dopamine release in terminal regions.
Accordingly, DRN serotonin cells recorded from the FBR group showed higher frequency and coefficient of variation. Therefore, we found changes in discharge pattern when compared to controls.

Taken together, our results suggest that chronic exposure to the PPARα agonist fenofibrate affects activity of VTA dopamine and DRN serotonin neurons. These advances might help understanding the mechanism whereby PPARα is implicated in the pathophysiology of neuropsychiatric disorders, and represent a novel promising therapeutic target.

P 20/09

ELEVATION OF KYNURENIC ACID LEVELS SUPPRESSES DELTA9-TETRAHYDROCANNABINOL-INDUCED EXCITATION OF MESOLIMBIC DOPAMINE AND PREFRONTAL CORTEX PYRAMIDAL NEURONS

Sagheddu Claudia[1], Melis Miriam[1], Muntoni Anna Lisa[2], Pistis Marco[1]

[1]Università degli Studi di Cagliari ~ Cagliari ~ Italy, [2]C.N.R. Neuroscience Institute ~ Cagliari ~ Italy

Delta9-tetrahydrocannabinol (Δ9-THC), the major psychoactive component of Cannabis extracts, like most drugs of abuse, enhances dopaminergic (DA) transmission by increasing both DA neuron firing rate and DA release in the nucleus accumbens shell (shNAc), an effect that underlies the rewarding and dependence-inducing effects of marijuana. We have recently demonstrated that elevations of brain levels of kynurenic acid (KYNA), an endogenous product of the normal metabolism of amino acid L-tryptophan, suppresses THC-induced behavioral and neurochemical effects, in rats and monkeys (Justinova et al. 2013).

To investigate how KYNA modulates THC-induced electrophysiological actions on DA neurons in the ventral tegmental area (VTA) and pyramidal neurons in the medial prefrontal cortex (mPFC), we carried out in vivo electrophysiological single cell recordings in anesthetized rats and ex vivo patch clamp experiments.

Neurons were selected as projecting to the shNAc by antidromic stimulation. According with previous studies, THC (0.3-2.4 mg/kg), increased firing activity of DA (137.1 ± 4.1 %) and mPFC (306.2 ± 75.6 %) cells projecting to the shNAc. To enhance brain levels of KYNA, the kynurenine-3-monooxygenase inhibitor, Ro 61-8048 (Ro, 30 mg/kg, i.p.) was administered 40 minutes before recordings. KYNA was suggested to act as a negative allosteric modulator of α7 nicotinic acetylcholine receptors (α7-nAChRs), therefore, to prevent Ro effects we administered a positive allosteric modulators of α7-nAChRs, PNU120596.

Consistent with microdialysis and behavioral studies, THC-induced increase in firing activity was completely abolished in DA (103.6 ± 3.3 %) as well in mPFC (119.4 ± 28.1) cells recorded from rats pretreated with Ro. Ex vivo patch clamp experiments confirmed that KYNA prevents THC-induced depression of excitatory post-synaptic potentials in DA neurons.
PNU120596 partially prevented the effects of Ro on mPFC pyramidal neurons, suggesting that the electrophysiological effects of KYNA might be dependent on α7-nAChR. The involvement of α7-nAChR was confirmed also with patch clamp experiments.

Patients seeking help for Cannabis dependence are increasing worldwide but specific pharmacological treatments are lacking. Together with recent neurochemical and behavioral studies, our results support the hypothesis that specific modulation of KYNA levels might represent an innovative therapeutic approach to treat Cannabis dependence.

P 21/09

EARLY ALTERATION OF HIPPOCAMPAL NEURONAL FIRING INDUCED BY ABETA42 OLIGOMERS IN ALZHEIMER'S DISEASE

Gavello Daniela[1], Allio Arianna[1], Calorio Chiara[1], Marie Hélène[2], Carbone Emilio[1], Marcantoni Andrea[1]

[1]University of Torino ~ Torino ~ Italy, [2]Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), CNRS (UMR7275) ~ Valbonne ~ France

Among the various hallmarks of Alzheimer's Disease (AD), the activation process of the "amyloid-cascade" is one of the most studied. It assumes that the accumulation of oligomers of amyloid beta peptides (Abeta), produced by the proteolytic processing of the amyloid precursor protein (APP), is the initiating event that triggers the progressive dismantling of synapses, neuronal circuits and networks. However, so far there are not yet clear data regarding any possible Abeta-induced impairment of neuronal excitability. Our previous studies in Tg2576 mice (Marcantoni et al., Pfluegers Arch 466:1437, 2014) indicate that neurons from lateral entorinhal cortex (LEC) exhibit an early impairment of their excitability profile. These mice are characterized by over-production of different Abeta peptides, like Abeta40, Abeta42, Abeta*56 and exhibit hyperphosphorylated tau, thus any neuronal impairment observed is not related to a single cause.

Being Abeta42 one of the peptides produced by APP processing known to induce early and severe neuronal function impairments, here we propose to test its effect on the Ca2+-dependent excitability profile of hippocampal neurons

To this purpose we performed intracellular Ca2+ measurements by confocal fluorescence microscopy and extracellular recordings of the hippocampal network excitability by means of microelectrode arrays (MEA)

Our preliminary experiments on cultured hippocampal networks reveal that pre-incubation of neurons with Abeta42 oligomers increases intracellular Ca2+ concentration. This effect is accompanied by a paradoxical firing inhibition. The study of the cause of the Abeta42 dependent Ca2+ dyshomeostasis let us to conclude that both ryanodine (RyrRs) and NMDA receptors (NMDARs) function are altered. When we focused on hippocampal network excitability, we indicated RyrRs as the main target of Abeta42, being their inhibition followed by a (partial) recovery of both firing frequency and synchronism. We also found that incubation of the BK channels inhibitor paxilline with Abeta42 oligomers antagonized the oligomers-induced inhibition of firing activity, indicating that BK channels may be a possible early target of AD. Finally, we show that the block of NMDA receptors is followed by an increased firing synchronization accompanied by a decreased firing frequency.

In conclusion, by focusing on the early effects of Abeta42 on Ca2+-dependent neuronal excitability, we identified three main direct or indirect targets: RyrRs, NMDARs and BK channels. Accordingly to previous reports, we further indicate RyrRs as critically involved in AD development. Thus, their inhibition may in turn be useful for identifying effective therapies that could enhance the life quality of AD patients.

P 22/09

GABAA- AND BENZODIAZEPINE RECEPTOR-MEDIATED INHIBITION OF HIPPOCAMPAL NEURONAL FIRING INDUCED BY BUD EXTRACTS FROM TILIA TOMENTOSA MOENCH

Allio Arianna, Calorio Chiara, Franchino Claudio, Carbone Emilio, Marcantoni Andrea

Department of Drug Science, University of Turin, Italy ~ Turin ~ Italy

Tilia tomentosa Moench bud extracts (TTBEs) are used in traditional medicine since more than fifty years as sedative compound. Different plants belonging to the Tilia genus have shown their efficacy in the treatment of anxiety but still little is known about the mechanism of action of their bud extracts.

Evaluate the action of TTBEs as anxiolytic and sedative compound on in vitro hippocampal neurons

The anxiolytic effects of TTBEs were assayed by testing the effects of these compounds on both the GABAA receptor-activated chloride current of hippocampal neurons and the synchronous activity of hippocampal networks by means of the patch-clamp technique and microelectrode-arrays (MEAs).

TTBEs acutely administered on mouse hippocampal neurons, activated a chloride current comparable to that measured in the presence of GABA (100 µM). Bicuculline (100 µM) and picrotoxin (100 µM) blocked about 90% of this current, while the remaining 10% was blocked by adding the benzodiazepine (BDZ) antagonist flumazenil (30 µM). Flumazenil alone blocked nearly 60% of the TTBEs-activated current, suggesting that TTBEs bind to both GABAA and BDZ receptor sites. Application of high-doses of TTBEs on spontaneous active hippocampal neurons grown for 3 weeks on MEAs blocked the synchronous activity of these neurons. The effects were mimicked by GABA and prevented by picrotoxin (100 µM) and flumazenil (30 µM). At minimal doses, TTBEs reduced the frequency of synchronized bursts and increased the cross-correlation index of synchronized neuronal firing.

Our data suggest that TTBEs mimic GABA and BDZ agonists by targeting hippocampal GABAergic synapses and inhibiting network excitability by increasing the strength of inhibitory synaptic outputs. Our results contribute toward the validation of TTBEs as effective sedative and anxiolytic compound.

P 23/09

LEVETIRACETAM DOWN-REGULATES THE EXPRESSION OF SEVERAL SYNAPTIC VESICLES PROTEINS IN RAT BRAIN CORTEX

Marcotulli Daniele, Fattorini Giorgia, Bragina Luca, Conti Fiorenzo

Department of Experimental and Clinical Medicine, Università Politecnica delle Marche ~ Ancona ~ Italy

Levetiracetam (LEV), a beneficial anti-epileptic drug (AED), is receiving growing interest in the therapy of various diseases, including dyskinesia, neuropathic pain, and Alzheimer disease. LEV is known to be an SV2A-binding molecule, thereby affecting synaptic transmission in an activity-dependent manner. However, LEV's mechanism of action is still unclear, and it is not known whether LEV has a preferential effect on GABAergic or glutamatergic synaptic terminals.

Our purpose was to investigate whether LEV treatment determines modifications in synaptic machinery release and whether these modifications involve glutamatergic and/or GABAergic terminals. Here we show that LEV treatment down-regulates the expression of several synaptic vesicle proteins, including vesicular glutamate and GABA transporters, in rat brain cortex.

Protein expression was evaluated by western blotting studies in animals treated with LEV (14 days, 54 mg/kg/day intraperitoneal) and controls. Vesicular transporters down-regulation was confirmed with quantitative confocal microscopy studies.

LEV treatment reduced expression of the following proteins: synaptotagmin 1 (to 76±4.44% of controls); synaptotagmin 2 (to 85.48%±3.21%); synaptotagmin 9 (to 79.43±4.27%); synapsin II (to 69.15±4.78%); synaptogyrin 1 (to 76.27±3.67%); synaptogyrin 3 (to 73.92±4.56%); VGLUT1 (to 68.81±5.24%), VGLUT2 (to 82.60±5.47%) and VGAT (to 77.33±4.77%). SV2A expression was not modified following LEV treatment. Vesicular proteins not regulated by LEV included SV2B, Rab3a, Rab3b, synaptophysin I, synapsin I, VAMP1, and VAMP2. Moreover, none of synaptic membrane proteins studied (STX1A, STX1B, SNAP23, SNAP25) were regulated by LEV.
Quantitative confocal microscopy studies showed a significant reduction of the density of VGLUT1+, VGLUT2+ and VGAT+ puncta and no significant differences of puncta size in LEV-treated animals.

These findings confirm that synaptic vesicles proteins are highly integrated and modifiable, and that their changes are crucial for synaptic and brain activity. Our results also suggest that LEV effectiveness, beyond SV2A-binding, involves GABAergic and glutamatergic synaptic vesicles modifications. These modifications are massive in some terminals, and not evident in others. Knowing the proteins whose expression is modified by LEV could help to better identify responder and non-responder patients. Furthermore, these proteins could be suitable novel therapeutic targets for epilepsies and other diseases.

P 24/09

TDP43 MODULATION BY THE GLUTAMATERGIC SYSTEM

Nutini Michele, Longone Patrizia

Fondazione Santa Lucia ~ Rome ~ Italy

The transactive response DNA-binding protein 43 (TDP-43), a DNA/RNA binding protein shuttling between nucleus and cytoplasm, is one of the major components of neuronal inclusions in sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) patients..
As a neuronal protein it feasible to infer that TDP-43 is modulated by synaptic activity. Indeed, recent data suggest that neuronal depolarization modulates TDP-43 translocation along the neuronal projections.

Previous works have focused on TDP43 function in neuronal cells, where KCL depolarization or BDNF application has shown to influence its translocation along axons and dendrites, suggesting a role for this protein in keeping the homeostasis of these neuron-specific compartments.
We meant to evaluate if the excitatory neurotransmitters could affect the subcellular localization of TDP-43 in cultured neurons. This feature is of particular interest due to the role that the glutamatergic system has in synaptic function and synaptogenesis and its involvement in the ALS neuronal demise. We focused on both the nuclear export process and the synaptic recruitment of the endogenous protein, following glutamate receptors activation.

To address these issues we prepared cortical and hippocampal cultures from mouse embryos, further treated with glutamate receptors (GluRs) agonists. Immunofluorescence and Western blots are the main techniques performed on such preparations.

We found a causal link between GluRs activation, with a major effect on the NMDA type, and TDP43 nuclear export and synaptic localization. We are currently studying the factors involved in these processes.

Our data suggest that TDP43 localization responds to glutamatergic stimuli at subtoxic concentrations, we believe that this interplay could unmask unprecedented TDP-43 features linked to the formation of neuronal networks and learning and memory processes.

P 25/09

CHOLINERGIC MODULATION OF MEDIAL SEPTUM AND HIPPOCAMPAL CA3 OUTPUTS IN VIVO

Griguoli Marilena[1], Zucca Stefano[2], Malezieux Meryl[2], Mulle Christophe[2]

[1]European Brain Research Institute "Fondazione Rita Levi-Montalcini" ~ Rome ~ Italy, [2]Interdisciplinary Institute for Neuroscience ~ Bordeaux ~ France

The hippocampus receives prominent innervation from the medial septum (MS) via the fimbria-fornix. Acetylcholine (ACh), released by MS projections, targets nicotinic and muscarinic receptors distributed in all hippocampal regions including CA3, particularly involved in new memory encoding. The relevance of the hippocampus and the CA3 region for memory processes and the importance of the cholinergic system for cognitive functions make it necessary to elucidate how ACh controls CA3 circuits in the intact network of a mammalian brain.

We aim at understanding how ACh released in the hippocampus controls the output of CA3 hippocampal neurons. Furthermore we aim at studying whether activation of cholinergic neurons modulates the firing of local MS circuit possibly affecting the output of CA3 through an indirect pathway.

We used an optogenetic approach combined with electrophysiological recordings from MS and CA3 neurons. Double-floxed inverted open reading frame (DIO) viral vectors were used to target the expression of ChR2-EYFP to the cholinergic neurons of the MS in ChAT-Cre mice. Whole cell patch clamp in acute slice and extracellular (local field potential, juxtacellular) recordings in anesthetized mice were obtained.

Whole cell recordings from EYFP+ cholinergic cells in acute slices showed that brief pulses of light (1 ms; 470 nm) reliably induced action potentials at different frequencies of stimulation. Local field potential including firing and oscillations (theta and gamma) in basal conditions and after light-based cholinergic stimulation at different frequencies were recorded in vivo. Activation of cholinergic neurons in the MS increased the firing frequency of CA3 neurons and was associated with an increase in the occurrence of theta oscillations. Juxtacellular recordings from CA3 pyramidal cells and interneurons showed that ACh increases the firing frequency of both cell types. Juxtacellular recordings from neurons in the MS showed that in a subpopulation of MS neurons the action potential triggered by light was followed by a significant inhibition of the spontaneous firing. The rest of MS neurons were either not responding or indirectly modulated by light showing a slow increase in the spontaneous firing.

The activation of cholinergic neurons changes the MS firing dynamics, possibly affecting hippocampal circuits indirectly. Further investigations are needed to dissect out the direct modulation by ACh released in CA3 region from the indirect one due to local changes in the MS circuit. This study will provide new insights on the functional role of ACh in modulating CA3 circuits involved in the rapid encoding of new information.

P 26/09

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CALRETININ INTERNEURONES IN THE MOUSE OLFACTORY BULB

Pignatelli Angela[1], Fogli Iseppe Alex[2], Batran Samah[1], Pighin Faride[1], Zucchini Elena[1], Scaduto Pietro[1], Belluzzi Ottorino[1]

[1]Università di Ferrara. Dipartimento di Scienze della Vita e Biotecnologie ~ Ferrara ~ Italy, [2]University of Davis, Dep. Neurobiology, Physiology and Behavior ~ Davis, Ca ~ United States

Within the olfactory bulb (OB), periglomerular (PG) cells consist of various types of interneurons, generally classified by their chemical properties such as neurotransmitter and calcium binding proteins. Calretinin (CR), calbindin D-28k, neurocalcin, parvalbumin and secretagogin are a group of calcium-binding proteins (CaBP) belonging to the EF-hand homologue family capable to bind intracellular calcium with dissociation constants in the micromolar range, all characterized morphologically and functionally diverse subclasses of PG cells in the OB. Compared to the very accurate morphological description available for the various PG cells subpopulations, functional data are rather unsatisfactory, mainly for the difficulty in recognising the different subtypes in living preparations.

The use of transgenic mice is progressively filling the knowledge gap for many CaBP-containing neurons in various areas of the CNS, but not for the olfactory bulb. Since in this structure the highest number of cells immunoreactive for Ca-BP was found for CR, it was of some interest to provide a comprehensive view of the electrophysiological features defining these cells.

An accurate identification of CR neurons was made possible by using of transgenic mice expressing eGFP under the CR promoter, and electrophysiological recordings were made in these cells using the patch-clamp technique in thin slices. Using ion substitution methods and specific blockers, we dissected the main voltage-dependent conductances present, obtaining a complete kinetic description for each of them.

The more peculiar property of these cells from the electrophysiological point of view is that they present only a single K-current, which is the IA - there is no trace of delayed rectifier or of Ca-dependent K-current. As a consequence, in response to prolonged depolarisations, after the inactivation of the A-current, these cells behave as a purely ohmic elements, showing no outward rectification. Other currents identified, isolated and fully characterised are two inward currents (INa and ICa) and an inward rectifier cationic current, Ih-type.

The singular complement of conductances present in these interneurons seems to be designed to enable the cell to integrate separate depolarizing inputs over long times, and to makes the encoding properties of the cells particularly sensitive to the prevailing membrane potential.

P 27/09

IDENTIFICATION OF DIFFERENT SPLICING VARIANTS OF KV7.4 POTASSIUM CHANNELS IN THE F11 NEURONAL CELL LINE

Medoro Alessandro, Soldovieri Maria Virginia, Vinciguerra Iolanda, Taglialatela Maurizio

University of Molise, Department of Medicine and Health Science ~ Campobasso ~ Italy

Kv7 channels (Kv7.1-5) are slowly activating/deactivating delayed-rectifier K+ channels expressed in a wide range of excitable cells, where they control their membrane potentials and appear as promising pharmacological targets for hyperexcitability diseases. Kv7 subunits display six transmembrane segments (S1-S6) and intracellular N- and C-termini. The C-terminus contains the binding site for many regulatory molecules, such as calmodulin. In the inner ear, Kv7.1 and Kv7.4 channels are critical for auditory function: indeed, mutations in these genes cause nonsyndromic sensorineural deafness type-2, an autosomal dominant form of progressive hearing loss. Although four different mouse Kv7.4 variants (vA-vD), each showing a specific functional modulation by calmodulin, have been reported in the mouse cochlea, little is known on the expression and function of different Kv7.4 variants in neurons.

In the present work, we aim to identify the Kv7.4 splice variants expressed in hybrid F11 cells, obtained by rat embryonic dorsal root ganglion (DRG) fused with mouse neuroblastoma cells.

The mouse Kv7.4 cDNA vA sequence was obtained from the ENSEMBL database, while the other sequences were manually reconstructed from protein sequences; by contrast, among the five rat variant sequences, vEn was extracted from the ENSEMBL, while vX1, vX2, vX3 and vX4 predicted sequences were extracted from the NCBI database. After sequence alignment with ClustalΩ, primers specific for each of the three mouse mutually exclusive exons (9, 10 and 11) and for the divergent regions at both N- and C-termini of rat Kv7.4 variants were engineered. RT-PCR experiments were performed on the cDNA retrotranscribed from F11 cells total RNA: the expression of a specific variant was assessed by the correspondence between the size or the presence/absence of PCR products versus those predicted.

The results obtained suggest that mouse Kv7.4 sequences are heterogeneous at the C-terminal domain, including part of the calmodulin binding site. By contrast, rat Kv7.4 variants appear to differ, not only at this C-terminal region, but also at the exon sequences corresponding to the N-terminus and S1 segment.

In conclusion, F11 cells express mouse vA, vB and vD and all predicted rat variants, although the expression of the vX4 shortest isoform remains uncertain. Based on the high homology between rat and mouse isoforms (~90%), further experiments on non-hybrid cells (mouse or rat cells) will lead to a more accurate identification of the Kv7.4 isoforms expressed in neuronal cells.

P 28/09

IMMUNOCHEMICAL DETECTION OF BDNF AND TRKB IN THE BRAIN OF PSYCHOGENETICALLY SELECTED ROMAN HIGH- AND LOW-AVOIDANCE RATS

Serra Maria Pina[2], Poddighe Laura[2], Boi Marianna[2], Piludu Maria Antonietta[1], Giorgi Osvaldo[1], Corda Maria Giuseppa[1], Quartu Marina[2]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, sez. di Neuroscienze, Università di Cagliari ~ Cagliari ~ Italy, [2]Dipartimento di Scienze Biomediche, sez. di Citomorfologia, Università di Cagliari ~ Monserrato (CA) ~ Italy

The outbread Roman High- (RHA) and Low-Avoidance (RLA) rat lines were psychogenetically selected for rapid versus poor acquisition of active avoidance, respectively, and differ in many behavioural traits that closely resemble the cardinal symptoms of depression. Beyond the monoamine hypothesis of depression, compelling evidence suggests that mood disorders are characterized by reduced neuronal plasticity. Consistently, it has been shown that exposure to stress and antidepressant treatment modulate the expression of neurotrophic molecules and their relevant receptors, and that these changes show an anatomical specificity.

To characterize the molecular and neuronal systems involved in the pathogenesis of depression and in the mechanism of action of antidepressant treatments, we investigate on the immunochemical occurrence of Brain-derived neurotrophic factor (BDNF) and its high affinity tyrosine-kinase receptor trkB, in selected areas of the RHA and RLA rat brain.

Western blot (WB) and immunohistochemistry. Densitometric analysis of immunostained WB protein bands and tissue sections was used to quantify differences between the two rat lines.

WB analysis indicates that relative levels of BDNF markedly differed only in the hippocampus, where they appeared lower by 58% in RLA vs RHA rats. As for trkB, its relative levels markedly differed in the prefrontal cortex and the hippocampus, where they were lower in RLA vs RHA rats, and in the caudate-putamen complex proper where, in contrast, they looked higher in RLA vs RHA rats. No statistically significant differences were seen in nucleus accumbens and ventral tegmental area.
In tissue sections, BDNF-like immunoreactive (LI) material labelled rare neuronal cell bodies and was mainly localized to proximal neuronal processes and varicose nerve fibers distributed in telencephalic cerebral cortex, hippocampus, amygdala, nucleus accumbens, caudate-putamen complex proper, thalamus and ventral tegmentum of the midbrain. trkB-like immunoreactivity, in contrast, was mainly localized to neuronal cell bodies and proximal processes, unevenly distributed in the telencephalic cerebral cortex, the hippocampus, and the ventral tegmentum of the midbrain. Densitometric analysis of immunostained brain sections revealed that differences amongst the two groups are consistent to a large extent with WB data.

As a whole, the finding of a different expression of BDNF and trkB in the RLA vs RHA rat brains implies the occurrence of an altered neuronal availability and/or responsiveness to BDNF in specific brain regions and may contribute to outline the molecular and morphological basis for the

distinct vulnerability to depression in the two rat lines.
This work was supported by grants from L.R. 07/2007, RAS project 2012.

P 29/09

STUDY OF THE MECHANISMS REGULATING THE LOCALIZATION OF NA+/CA2+ EXCHANGER 3 IN MITOCHONDRIA AND ITS PATHOPHYSIOLOGICAL IMPLICATIONS

Fiorillo Stefania, Scorziello Antonella, Molinaro Pasquale, Sisalli Maria Josè, Annunziato Lucio

Division of Pharmacology, Department of Neuroscience, Reproductive and Odontostomatological sciences, School of Medicine, University of Naples "Federico II" ~ Pansini, 5, Naples 80131 ~ Italy

NCX3 is the only Na+/Ca2+ exchanger (NCX) isoform that is localized on plasma membrane and outer mitochondrial membrane (OMM) of neurons. Mitochondrial NCX3 plays an important role in mitochondrial calcium homeostasis where it participates in Ca2+ handling from the OMM through an AKAP121-anchored signaling complex, thus promoting cell survival during hypoxia. Interestingly, the amount of NCX3 localized on the OMM of neurons decreases during oxygen glucose deprivation and is restored after re-oxygenation (OGD-Rx), suggesting the presence of a regulatory mechanism in NCX3 cellular sorting.

The aim of our study is to investigate the regulatory mechanisms of NCX3 localization in mitochondria under physiological and pathophysiological conditions.

The bioinformatic analysis of NCX3 amino acid sequence was performed by means of TargetP1.1 software to identify putative mitochondrial localization signals. The amino acid alignment of the plasma membrane isoforms, NCX1 and NCX2, and the mitochondrial isoform NCX3 was performed by CrustalW algorithm. Chimeric proteins between NCX3 and NCX1 sequences and NCX3 mutants were generated by means of site-directed mutagenesis. All cDNA were stably expressed in baby hamster kidney cells. Differential extracts of membrane, cytosolic and mitochondrial proteins were analyzed by Western blot to identify which NCX chimera or mutant contains the molecular determinants recognized by the mitochondrial outer membrane import system.

Results demonstrated that there are several molecular determinants spread along the NCX3 protein that are responsible for NCX3 localization on OMM. These signals are localized in the C-terminal region of the cytosolic f-loop (718-756aa), and in the alpha1 (Leu117, Gly 124 and Ileu126) and alpha2 (Phe808, Leu823 and Val826) repeats of NCX3 amino acid sequence. Indeed, the insertion of these identified regions of NCX3 on NCX1 sequence, by substitution, was able to localize the non-mitochondrial protein NCX1 on mitochondria.

These findings could provide us with more information about the recognition mechanism of NCX3 on the OMM through the mitochondrial outer membrane import system that could be responsible of different NCX3 sorting in cells under different conditions. Then, next steps will be to identify which member of the translocase of outer mitochondrial membrane (TOM) protein complex, between the main subunits TOM20, TOM22 and TOM70, is involved in the recognition and transport of NCX3 on the OMM and to study the regulatory mechanism involved in NCX3 sorting also under pathophysiological conditions, such as in neurodegenerative diseases.

P 30/09

BIOCHEMICAL, MORPHOLOGICAL, AND PHARMACOLOGICAL EVIDENCE FOR KV7.4 CHANNELS EXPRESSION IN NEURONAL MITOCHONDRIA

Manocchio Laura[1], Vinciguerra Iolanda[1], Soldovieri Maria Virgina[1], Paventi Gianluca[1], Ambrosino Paolo[1], Calderone Vincenzo[2], Passarella Salvatore[1], Taglialatela Maurizio[1]

[1]University of Molise, Department of Medicine and Health Science ~ Campobasso ~ Italy, [2]University of Pisa, Department of Pharmacy ~ Pisa ~ Italy

Potassium channels in the inner mitochondrial membrane (mitoK channels) participate in ischemic preconditioning, namely in the ability of sublethal insults to protect against subsequent potentially lethal stimuli. In neurons, among the best studied mitoK channels are mitoKATP and mitoBK channels. The Kv7 family of voltage-gated potassium channels is an additional gene family including five members (Kv7.1-Kv7.5), each showing a specific cellular distribution and functional role. These channels have been shown to play a relevant role in in vitro and in vivo models of neuroprotection against anoxic/ischemic events.

Therefore, the aim of the present study has been to investigate the possible presence of Kv7 channels in neuronal mitochondria.

To this aim, expression of transcripts and proteins for Kv7 members was investigated by RT-PCR and western-blotting, respectively, in F11 cells, a hybrid cell line obtained by the fusion of rat embryonic dorsal root ganglion cells with mouse neuroblastoma cells. Western blotting experiments were also performed in subcellular fractions. Finally, mitochondrial membrane potential ($\Delta\Psi$) was monitored in isolated mitochondria by the safranin O fluorimetric method; a decrease in the safranin O fluorescence indicates an increase in $\Delta\Psi$.

RT-PCR experiments revealed that the mRNAs encoding for all Kv7 subunits were expressed in F11 cells; however, Western-blotting experiments only detected the expression of Kv7.4 subunits, which were particularly concentrated in mitochondria. $\Delta\Psi$ monitoring experiments revealed that externally added K+ decreased $\Delta\Psi$ with saturable kinetics, consistent with channel-mediated transport. Kinetic analysis of mitochondrial K+ transport in the presence and absence of the mitoKATP blocker glybenclamide (20 µM) revealed the presence of at least two K+ transport mechanisms; consistent with this, glybenclamide decreased but did not eliminate mitochondrial K+ transport. To investigate the possible contribution of Kv7 channels, the effects of Kv7 activators (retigabine) or inhibitors (XE991) on 20 mM KCl-induced mitochondrial depolarization were studied. The results obtained revealed that the rate of 20 mM KCl-induced mitochondrial depolarization was reduced by XE-991 (20 µM) and enhanced by retigabine (10 µM). More importantly, the stimulatory effect of retigabine was fully reversed by XE-991, while it was unaffected by glybeclamide.

Altogether, these results demonstrate that neuronal mitochondria contain Kv7.4 channels playing a critical role in mitochondrial K+ transport. Future experiments will assess whether these novel mechanisms might be implicated in neuroprotection triggered by preconditioning.

P 31/09

BEHAVIORAL AND ELECTROPHYSIOLOGICAL ASPECTS OF NEOCORTICAL CB1 SPECIFIC KO MICE REVEAL A MURINE MODEL OF NEUROPATHIC PAIN

Giustizieri Michela[1], Marrone Maria Cristina[1], Marsicano Giovanni[2], Marinelli Silvia[1]

[1]EBRI ~ rome ~ Italy, [2]University of Bordeaux ~ Bordeaux ~ France

Neuropathic pain (NP) is a clinical syndrome characterized by peripheral and central components and by the alteration of sensory perception such as an increased response to painful stimuli (hyperalgesia) and pain perception for not noxious stimuli (allodynia). Compelling evidence demonstrates that the endocannabinoid signalling regulates peripheral and central pain component due to the high density of cannabinoid receptors type 1 (CB1R) in both sensory fibers and central areas controlling pain sensations.

Dissect the contribution of specific neuronal CB1R expression in both nociceptive and cognitive-emotional components of NP.

Mice: adult male C57BL/6J, CB1+/+ (WT), CB1 KO-selectively targeting GABAergic (GABA) neurons (GABA-CB1-KO)/ glutamatergic (Glu) neurons (Glu-CB1-KO) or both GABA- GLU neurons (GABA/Glu-CB1-KO). These mice were tested in control (naïve and sham) at different time points after the chronic constriction injury (CCI; from 3 to 41 days).
Murine model of NP: CCI is made up of three ligatures of the paw sciatic nerve. Mechanical allodynia was assessed by using the aesthesiometer.
Field potential recordings : Anterior Cingulate Cortex (ACC) coronal slices from adult WT and specific CB1KO, both naïve and CCI mice. Field recordings from layer 2/3 pyramidal neurons and stimulation site in layer V. long-term potentiation (LTP) was induced by Theta Burst Stimulation (TBS).
Immunofluorescence Technique: slices containing ACC were incubated with primary antibodies and coupled with secondary antibodies by using indirect staining fluorescence method. Sections were examined with a four laser confocal microscope.

Mice suffering from NP (CCI mice) resulted in a selective impairment of mechanical nociceptive threshold in the ipsilateral paw that was maintained for at least 41 days; when compared to C57bl6j, CB1+/+ mice showed a bigger mechanical nociceptive sensibility, that was maximally evident in mice lacking CB1R selectively on GABA and glutamatergic forebrain neurons.
TBS induced LTP of the ACC of naïve WT mice. Both LTP paradigm and CB1R expression were significantly reduced in CCI mice. Similarly, LTP was reduced in WT cortical slices treated with the CB1R antagonist AM 251, as well as in normal ACSF perfused sections from GABA/Glu-CB1-KO mice. In contrast LTP was not impaired in both specific Glu-CB1-KO and GABA-CB1-KO slices.

Our in vivo and in vitro data suggest that mice specifically lacking forebrain CB1R in both Glu and GABA neurons have an altered sensory perception and behave as animals suffering from NP, underlying the crucial role of brain CB1R in the process of incoming sensory information.

P 32/09

ALPHA-SYNUCLEIN IMPAIRS LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION OF STRIATAL CHOLINERGIC INTERNEURONS TARGETING THE GLUN2D-EXPRESSING NMDA RECEPTOR.

De Iure Antonio[3], Tozzi Alessandro[3], Durante Valentina[3], Quiroga--Varela Ana[3], Tantucci Michela[3], Mazzocchetti Petra[2], Di Filippo Massimiliano[3], Costa Cinzia[3], Spaccatini Cristiano[3], Gardoni Fabrizio[1], El--Agnaf Omar[4], Paolo Calabresi[3]

[1]University of Milan ~ Milan ~ Italy, [2]Fondazione Santa Lucia IRCCS ~ Rome ~ Italy, [3]University of Perugia ~ Perugia ~ Italy, [4]United Arab Emirates University ~ Al Ain ~ United Arab Emirates

Parkinson's disease (PD) is characterized by a large and progressive degeneration of nigral dopaminergic neurons, which causes dramatic motor and cognitive dysfunction, and is characterized by the presence of nigral Lewy bodies, intracellular inclusions whose main constituent is alpha-synuclein. However, the synaptic mechanisms underlying the behavioural and motor alterations induced by early selective over-expression of nigral alpha-synuclein are still a matter of debate.

We studied the effect of alpha-synuclein application on long-term potentiation (LTP) of synaptic plasticity in rat striatal slices. Moreover, we assessed whether alteration of LTP correlated with altered performance of rats in a motor learning test.

We performed patch-clamp recordings of medium spiny neurons (MSNs) and cholinergic interneurons (ChIs) and measured the NMDA-mediated excitatory postsynaptic currents (EPSCs), as well as the LTP, in control conditions and in slices treated with alpha-synuclein. We used the active-avoidance learning test to explore possible deficits linked to the altered synaptic plasticity of striatal neurons.

We found that low doses of alpha-synuclein abolished the LTP of ChIs but not of MSNs and that this effect was mediated by the GluN2D-containing NMDA receptor, selectively expressed in ChIs. We also found that rats injected with QNZ-46, a selective inhibitor of the GluN2D-containing NMDA receptor, presented reduced ability to perform the active-avoidance learning test.

We suggest that striatal cholinergic dysfunction, induced by a direct interaction between alpha-synuclein and GluN2D-expressing NMDA receptors, represents a precocious biological marker of PD.

P 33/09

TM4SF2 KNOCKOUT MICE DISPLAY SYNAPTIC DYSFUNCTIONS AND DEFICITS IN ASSOCIATIVE MEMORY

Murru Luca[1], Folci Alessandra[1], Vezzoli Elena[2], Moretto Edoardo[1], Bassani Silvia[1], Longatti Anna[1], Bianchi Veronica[3], D''Adamo Patrizia[3], Francolini Maura[2], Passafaro Maria[1]

[1]CNR Institute of Neuroscience University of Milan ~ MILANO ~ Italy, [2]University of Milan, Department of Medical Biotechnology and Translational Medicine ~ MILANO ~ Italy, [3]Dulbecco Telethon Institute at Division of Neuroscience, San Raffaele Scientific Institute ~ MILANO ~ Italy

Mutations in TSPAN7, member of the tetraspanin protein superfamily, are implicated in some form of X-linked intellectual disabilities. TSPAN7 is encoded by TM4SF2 gene located on Xp11.4. We found that TSPAN7 is involved in maturation of glutamatergic synapses and AMPA receptors trafficking (Bassani et.al 2012).

Recently, we generated a knock out (KO) animal model for TM4SF2 in order to study the role of TSPAN7 on synapses in vivo.

Methods used for this study:
Electron microscopy to perform ultrastructural analysis of synapses in hippocampal CA1 region; Molecular biology techniques to study synaptic markers expression in KO and WT mice; Golgi-cox staining to study neuronal morphology; electrophysiologal techniques to study TSPAN7 involvement with neuronal function in the hippocampus, with particular attention at the excitatory synapses; Behavioral tests to investigate the role of TSPAN7 on mice behavior.

Here we showed that the density of spines and the length and thickness of the PSDs was significant reduced in TM4SF2 KO mice compared to WT mice.
Functional experiments revealed that TM4SF2 loss of function causes a reduction in mEPSCs frequency, area and amplitude in CA1 pyramidal neurons with no changes in the paired pulse ratio (PPR), suggesting a strictly involvement of the post-synaptic compartment. We also observed a dramatic impairment of long term potentiation (LTP) in CA1 pyramidal neurons.
In association with these synaptic alterations the TM4SF2 KO mice presented hyperactivity and defects in associative memory during fear conditioning paradigm.

In conclusion, these data suggest that TSPAN7 has a key role for the correct formation, maturation and function of the excitatory synapse in mouse hippocampus and for associative memory behaviors.

P 34/09

ALPHA-SYNUCLEIN PRODUCES NMDA-MEDIATED STRIATAL SYNAPTIC DYSFUNCTION

Durante Valentina[1], Tozzi Alessandro[1], De Iure Antonio[1], Quiroga-Varela Ana[1], Tantucci Michela[1], Mazzocchetti Petra[2], Spaccatini Cristiano[1], Di Filippo Massimiliano[1], Costa Cinzia[1], El-Agnaf Omar[3], Calabresi Paolo[1]

[1]University of Perugia ~ Perugia ~ Italy, [2]Fondazione Santa Lucia IRCCS ~ Rome ~ Italy, [3]United Emirates University ~ Al Ain ~ United Arab Emirates

Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system (CNS) associated with motor impairment caused by the progressive degeneration of dopaminergic neurons of the substantia nigra. Dopamine (DA) controls motor behavior by regulating striatal synaptic function. Loss of striatal synaptic plasticity is the key pathological consequence of the massive degeneration of dopaminergic neurons. In fact, synaptic plasticity of striatal medium spiny neurons (MSNs) is dependent on endogenous DA. PD is also characterized by accumulation of intracellular inclusions, named Lewy bodies, in the substantia nigra and other brain areas. Lewy bodies are mainly composed of alpha-synuclein, neuronal presynaptic protein of 140 aminoacid largely expressed in the CNS. Alpha-synuclein is genetically and neurophatologically associated to PD, in fact, mutations of this protein or increased expression of wild-type alpha-synuclein result in rare form of PD characterized by early onset and autosomal dominant inheritance.

We hypothesized that alpha-synuclein affected neuronal signaling by targeting DA release or the receptor function that is directly involved in MSN synaptic transmission, such as the NMDA and AMPA glutamate receptors.

Thus, we investigated the synaptic effects of oligomeric alpha-synuclein on striatal long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission performing patch-clamp experiments of MSNs recorded from striatal rat slices treated with recombinant human alpha-synuclein and in control conditions. Moreover, we used an amperometric technique to assess whether alpha-synuclein was able to impair DA release in striatal slices.

We found that in MSNs 30 nM of alpha-synuclein oligomers reduced the LTP by targeting the GluN2A-containing NMDA receptor while showing no effect on the LTD. Notably, lower (3 nM) alpha-synuclein concentrations were not able to block the LTP of MSNs whereas it affected that of striatal cholinergic interneurons. We also found that the effect of alpha-synuclein on MSNs was not dependent on alteration of DA release. In fact, amperometric measurements showed only a small effect of alpha-synuclein in reducing striatal evoked DA release. Moreover, acute application of exogenous DA failed to restore the altered LTP.

These results indicate that accumulation of oligomeric striatal alpha-synuclein, which can be found in different animal models of PD and in patients, may produce impairment of striatal function by directly targeting in MSNs the GluN2A-expressing NMDA receptor and LTP.

P 35/09

TUMOR NECROSIS FACTOR RELATED APOPTOSIS INDUCING LIGAND (TRAIL) ATTENUATES THE EXPRESSION OF THE NA+/CA2+ EXCHANGER (NCX3) IN DIFFERENTIATED SH-SY5Y NEURONAL CELLS

Valerio Oriana[1], Di Benedetto Giulia[2], Lariccia Vincenzo[1], Bernardini Renato[2], Amoroso Salvatore[1], Cantarella Giuseppina[2]

[1]Department of Biomedical Sciences and Public Health, School of Medicine, Università Politecnica delle Marche, Ancona ~ Ancona ~ Italy, [2]Department of Biomedical and Biotechnological Sciences, University of Catania ~ Catania ~ Italy

TRAIL, a proinflammatory/proapoptotic cytokine belonging to the TNF superfamily, is endowed with prominent neurotoxicity. In addition, disruption of neuronal calcium homeostasis results in injury. The NCX3 Na+/Ca2+ exchanger, which plays a relevant role as regulator of intracellular Ca2+ levels, protects neurons from lethal damage. NCX3 expression is induced by nerve growth factor (NGF), through activation of its TrkA receptors and associated kinases, and a reduction in its expression may thus contribute to neurodegeneration.

Here, the hypothesis whether the loss of NCX3 function was related to increased activity of TRAIL was verified in neuronal cell cultures.

Differentiated human neuroblastoma SH-SY5Y cells were incubated with TRAIL for up to 48 h. NCX3 protein expression, along with those of pTrkA, pErk1/2 and pAkt, was studied by means of Western blot analysis on cell lysates collected after 6, 16, 24 and 48 h of incubation.

NCX3 expression decreased in a time-dependent fashion in the presence of TRAIL. Moreover, calpain-mediated cleavage of NCX3 was potentiated by TRAIL. Western blot analysis revealed that pTrkA expression was initially increased in SH-SY5Y cells treated with TRAIL for 6 and 16h, whilst it declined thereafter, and almost disappeared after 48h. A similar pattern was observed for both pErk1/2 and pAkt.

Results suggest that the increase of TRAIL expression occurring during neuronal damage correlates with down-regulation of the NGF signalling, as well as with attenuation of NCX3 expression. The TRAIL system could thus represent a potential target for prevention of neuronal damage related to dysfunction of NCX3.

P 36/09

AGOMELATINE TREATMENT INDUCES SPECIFIC AND TIME-DEPENDENT MODULATION OF RAT HIPPOCAMPAL MIRNAS AND RELATED TARGET GENES

Seguini Mara[1], Tardito Daniela[1], Rota Veronica[1], Merelli Ivan[2], Corrada Dario[3], Milanesi Luciano[2], Racagni Giorgio[1], Popoli Maurizio[1]

[1]Laboratory of Neuropsychopharmacology and Functional Neurogenomics, Department of Pharmacological and Biomolecular Sciences and Center of Excellence on Neurodegenerative Diseases ~ Milano ~ Italy, [2]Institute for Biomedical Technologies, National Research Council ~ Segrate, Milano ~ Italy, [3]Department of Earth and Environmental Sciences, University of Milano-Bicocca ~ Milano ~ Italy

MicroRNAs (miRNAs) are small, non-coding RNAs with a key role in regulation of gene expression at post-transcriptional level. miRNAs play a crucial role into signaling and network modulation in almost every cellular process, including neuronal development and homeostasis. Recent investigations suggested that dysregulations in miRNA expression may be involved in mental disorders pathophysiology and psychotropic drugs action.

Aim of this study was to verify whether treatment for different time lengths (3/7/21 days) with the antidepressant agomelatine could influence hippocampal miRNome expression profile and related target genes.

Rats were treated with vehicle or agomelatine (40 mg/kg/day i.p, 5 p.m.) for 3, 7 or 21 days. miRNA expression analysis was conducted by using TaqMan Array Rodent MicroRNA A+B Cards Set v3.0. In order to identify miRNA putative target genes and molecular pathways potentially involved, bioinformatic analyses were performed by integrating and filtering the results of different miRNA target prediction algorithms, followed by annotation analyses with Gene Ontology subcategories. Expression levels of selected putative target genes were measured by means of qRT-PCR and western blot analyses.

The expression analysis showed that hippocampal miRNome was significantly modulated at all time points assessed. In particular, 34 miRNAs were modulated after 3 days of treatment; 22 miRNAs after 7 days, and only 6 miRNAs were modified after 21 days. Bioinformatic analysis highlighted enrichment of miRNA targets in different pathways, some related to neuronal functions (including some previously associated to both depression pathophysiology and antidepressant action) and others, particularly after 3 and 7 days, to epigenetic mechanisms. Expression analysis of putative target genes confirmed for some of them a direct relationship to miRNA changes induced by agomelatine treatment. Indeed, we found significant and time-dependent modifications in the mRNA and/or protein levels of Dnmt1 and Hdac1 after 3/7 days, D1a, Grm2 and Hcrtr2 after 7 days, and BCl6 and D1a after 21 days of treatment.

Our results show that agomelatine can induce early and time-dependent modifications in rat hippocampal miRNome, with the main effect after 3 days of treatment. The bioinformatic analysis and the changes found in the expression of some target genes suggest the modulation of coordinate and fine-tuned specific functional networks involved in epigenetic mechanisms,

neuroplasticity and neurotransmission. Although further work is needed to get further insight, these results suggest that miRNA might be early mediators of agomelatine action.

P 37/09

ROLE OF ATP P2Y1 RECEPTOR IN THE RAT DENTATE GYRUS AFTER A SEVERE ISCHEMIC INSULT

Fusco Irene[3], Coppi Elisabetta[1], Maraula Giovanna[3], Lana Daniele[1], Giovannini Maria Grazia[1], Mello Tommaso[2], Galli Andrea[2], Pedata Felicita[3], Pugliese Anna Maria[3]

[1]2Dept. of Health Sciences, Clinical Pharmacology and Oncology Unit, University of Florence ~ Firenze ~ Italy, [2]3Dept. of Experimental and Clinical Biomedical Sciences, University of Florence ~ Firenze ~ Italy, [3]1Dept. of Neuroscience, Psychology, Drug Research and Child Health, Neurofarba, Division of Pharmacology and Toxicology, University of Florence ~ Firenze ~ Italy

P2Y1 receptors (P2Y1R) are widely expressed in the brain, including the dentate gyrus (DG), on both neurons and glial cells. Multipotent neural stem cells are present in the subgranular zone (SGZ) of the DG. They are able to proliferate and differentiate into neurons, astrocytes and oligodendrocytes in response to hypoxic-ischemic injury.

The purpose of our research was to study the contribution of P2Y1R to the recovery of neurotransmission and to the modulation of proliferative and maturational responses in the DG, in acutely isolated hippocampal slices.

Extracellular field excitatory post-synaptic potentials (fEPSPs) in granule cells of the DG were recorded from rat hippocampal slices by using electrophysiological technique. For immunohistochemical analysis, proliferating cells in the SGZ were detected by using the DNA replication marker 5-Bromo-2′-deoxyuridine (BrdU), a thymidine analog which incorporates into the DNA of all cells during the S-phase. To determine the phenotype of the newly born cells, doublecortin (DCX), an immature neuronal marker, was used.

A severe period of oxygen and glucose deprivation (OGD, 9 min duration) in acute rat hippocampal slices induced the appearance of anoxic depolarization (AD), a clear sign of tissue damage, and the irreversible block of synaptic activity in all the slices (n=26) examined. The selective P2Y1R antagonist MRS2179 (10 μM, n=23) prevented the appearance of AD, allowing an almost complete fEPSP recovery (96.0±12.5%, calculated 50 min from the end of OGD versus 4.0±4.5%, n=26, found in OGD-untreated slices). Data indicate that P2Y1R contribute to the early damage induced by OGD in the DG likely contributing to glutamate-induced excitotoxic effects that causes irreversible synaptic failure after severe OGD.
In hippocampal slices prepared from BrdU-treated rats and incubated with the immature neuronal marker DCX, the number of BrdU+ cells of the SGZ was significantly decreased 6 hours after OGD, but returned to control values 24 hours thereafter, when a significant increase of DCX immunofluorescence was also observed. MRS2179 significantly decreased the number of BrdU+ cells 24 hours after OGD.

Since it has been demonstrated that P2Y1R stimulation promotes cell proliferation in the SGZ niche, it is likely that the antagonism of P2Y1R in the DG, at times later from OGD, reduces cell proliferation.

P 38/09

ETHANOL MODULATES HYPERPOLARIZATION-ACTIVATED CATION CURRENTS (IH) IN RAT HIPPOCAMPAL CA3 PYRAMIDAL NEURONS: INVOLVEMENT OF THE AC/CAMP/PKA INTRACELLULAR PATHWAY

Licheri Valentina[3], Talani Giuseppe[1], Biggio Giovanni[2], Sanna Enrico[2]

[1]Institute of Neuroscience, National Research Council ~ Monserrato, 09124 Cagliari ~ Italy, [2]Department of Life and Environmental Sciences, Section of Neuroscience and Anthropology, Institute of Neuroscience, National Research Council, Monserrato, 09124 Cagliari ~ Monserrato, 09124 Cagliari ~ Italy, [3]Department of Life and Environmental Sciences, Section of Neuroscience and Anthropology, University of Cagliari, Monserrato, 09124 Cagliari ~ Monserrato, 09124 Cagliari ~ Italy

The hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are activated by membrane hyperpolarization and mediate cationic and inward currents, called Ih. The activity of HCN is regulated by cyclic nucleotides, such as cyclic adenosine monophosphate (cAMP). Furthermore, D1 receptors activation modulates the function of HCN through the AC/cAMP/PKA pathway. HCN are involved in the control of resting membrane potential, dendritic integration, membrane excitability and in the regulation of presynaptic neurotransmitters release. Recent evidences reported that the function of HCN in hippocampal interneurons is regulated by ethanol (EtOH) suggesting that these channels may represent an additional target for EtOH central action.

Since HCN are highly expressed in CA3 glutamatergic neurons, we have characterized the action of EtOH on Ih in these hippocampal cells and evaluated the possible involvement of the AC/cAMP/PKA intracellular pathway.

EtOH and other drugs were bath applied and neuronal responses were recorded under voltage-clamp conditions in acute coronal slices from male Sprague-Dawley rat brains containing the hippocampal formation.

EtOH modulates Ih in a biphasic manner, with of 20 mM EtOH enhancing Ih amplitude, while higher concentrations (60-80 mM) producing an inhibitory effect. This biphasic action of EtOH is reflected by changes in carbachol-induced firing rate and dendritic synaptic integration of AMPA receptor-mediated EPSPs. The modulatory effects of EtOH were completely antagonized by the bath perfusion of the selective inhibitors of adenylyl cyclase and PKA, DDA (10 µM) and H89 (10 µM) respectively. Moreover forskolin, an activator of AC, at a concentration of 0.1 µM increased the amplitude of Ih, while 30 µM produced an opposite effect, reducing the same parameter.

Altogether the results demonstrate that EtOH modulates the function of HCN channels in the rat CA3 pyramidal neurons, mainly through the AC/cAMP/PKA intracellular pathway. Overall these findings suggest that the biphasic modulatory action of EtOH on HCN channels may contribute to the effects of EtOH on the excitability of the hippocampal formation.

P 39/09

FOOD RESTRICTION IN RATS IS ASSOCIATED WITH AN ENHANCED GLUTAMATERGIC SYNAPTIC PLASTICITY IN THE HIPPOCAMPAL CA1 FIELD: INVOLVEMENT OF CB1 RECEPTORS

Talani Giuseppe[1], Licheri Valentina[2], Biggio Francesca[2], Locci Valentina[3], Mostallino Maria Cristina[1], Cocco Laura[2], Firino Laura[2], Sitzia Giacomo[2], Biggio Giovanni[4], Sanna Enrico[4]

[1]Institute of Neuroscience, National Research Council, 09042 Monserrato, Cagliari, Italy. ~ Monserrato, 09124 Cagliari ~ Italy, [2]Department of Life and Environmental Sciences, Section of Neuroscience and Anthropolgy, University of Cagliari, ~ Monserrato, 09124 Cagliari ~ Italy, [3]Department of Biomedical Science, University of Sassari ~ Sassari ~ Italy, [4]Department of Life and Environmental Sciences, Section of Neuroscience and Anthropology, University of Cagliari, Institute of Neuroscience, National Research Council, 09042 Monserrato, Cagliari ~ Monserrato, 09124 Cagliari ~ Italy

The endogenous endocannabinoid system plays a crucial role in regulating appetite and feeding behavior in mammals as well as working memory and reward mechanisms. Previous work demonstrated that food restriction (FR) in rats involves changes in expression and function of CB1Rs in the PFC with consequent altered GABAergic inhibition and DA output. Such adaptive changes, in turn, may be consistent with a learning process that may involve different brain regions such as the hippocampus.

In order to elucidate the possible role of cannabinoid type-1 receptors (CB1Rs) in the regulation of hippocampal plasticity and glutamatergic transmission we applied a protocol of (FR) by which male rats have a limited (2-h daily) access to food, for a total duration of 3 weeks.

In order to evaluate the glutamate transmission and long-term synaptic plasticity in the hippocampus, patch-clamp and extracellular field recordings were carried out in acute hippocampal slices. Western blot analysis was used to evaluate the amount of hippocampal CB1Rs and BDNF protein. Barnes maze test was used to evaluate the possible changes in spatial learning and memory.

FR showed a higher LTP at hippocampal CA1 excitatory synapses with a parallel increase in the probability of presynaptic glutamate release when compared to animal fed ad libitum. FR was also associated with a decreased inhibitory effect of the CB1R agonist WIN55,212-2 on glutamatergic fEPSPs, together with a decrease in hippocampal CB1R protein expression. In addition, hippocampal BDNF protein levels and mushroom dendritic spine density were significantly enhanced in FR rats. These result are supported by a decrease in the time spent to find the target hole in the Barnes maze of FR rats compared to controls, suggesting that FR is associated with an increase in spatial memory performance. Moreover, the higher LTP found in FR rats could be mimicked by a sub-chronic treatment (7 days) of controls rats with the selective CB1R antagonist SR141716.

Our results show that FR induces an increased levels of neurotrophic factors and LTP formation and are consistent with the hypothesis that FR in rats could have ameliorating effects on cognitive functions related to the hippocampal formation, an effect that appeared correlated to a decrease in

expression/function of CB1 receptors at excitatory synapses.

The present work has been funded by the Regione Autonoma della Sardegna, Progetti di Ricerca di Base, Bando 2010 and the Sardinian Agency for R&TD (Sardegna Ricerche).

P 40/09

EFFECT OF PSYCHOSTIMULANT DESIGNER DRUGS ON BRAIN NEUROTRANSMITTERS

Kaminska Katarzyna, Noworyta--Sokolowska Karolina, Jurczak Alexandra, Górska Anna, Golembiowska Krystyna

Institute of Pharmacology of the Polish Academy of Sciences ~ Kraków ~ Poland

Designer drugs, also known as „legal highs", are synthetic compounds developed to provide similar effects to illicit drugs of abuse, which are not subjected to legal control. Synthetic drugs are classified roughly, based on their chemical formula, into phenethylamines, tryptamines, and piperazines. Although designer drugs still have the reputation of being safe, several experimental studies in rats and humans indicated risks including life-threatening serotonin syndrome, hyperthermia, neurotoxicity, and abuse potential.

The least investigated and most dangerous synthetic components found in the designer mixtures are phenylalkylamines: para-methoxyamphetamine (PMA), para-methoxymethamphetamine (PMMA) and synthetic cathinone: mephedrone. Our study is aimed at examining the effects of the above-mentioned on tissue content of dopamine (DA),serotonin (5-HT) and their metabolites in various regions of rat brain.

Rats were injected intraperitoneally with PMA, PMMA at doses of 5 and 10 mg/kg while mephedrone was given at 10 and 20 mg/kg. 3 hrs after administration animals were decapitated and various brain regions regions were separated. Tissue samples were homogenized in 0.1 M $HClO_4$, filtered and assayed using HPLC with electrochemical detection.

Mephedrone markedly increased tissue content of DA and its metabolites DOPAC and HVA in nucleus accumbens (NAC), striatum (STR) and hippocampus (HP), while the level of DA and DOPAC was decreased in rat frontal cortex (FCx). PMA increased DA, while decreasing DOPAC level in NAC, STR and FCx. Similarly, PMMA enhanced DA and decreased DOPAC level in NAC and STR, attenuating the level of both in FCx. Mephedrone did not affect 5-HT and 5-HIAA content in NAC and STR, but decreased 5-HT and increased 5-HIAA level in FCx and Hp. PMA increased 5-HT and 5-HIAA level in NAC and FCx, while PMMA increased only 5-HT tissue level and decreased 5-HIAA level in NAC, STR and FCx.

These data indicate various pharmacological profiles of studied drugs, despite that they all inhibit DA and 5-HT transporters in vitro, in similar range of concentration. Mephedrone seems to stimulate synthesis of DA in areas rich in dopaminergic innervation. On the other hand, it increases 5-HT turnover in cortical and hippocampal brain regions. PMA and PMMA seem to inhibit DA turnover in cortical and subcortical brain regions. PMA stimulates, while PMMA inhibit cortical and sub-cortical 5-HT neurons. Our results suggest a serious impact of studied psychostimulants on dopaminergic and serotonergic neurotransmission in the rat brain.

P 41/09

EFFECT OF SUB-CHRONIC ADMINISTRATION OF MDMA AND CAFFEINE ON MONOAMINE TRANSMITTERS AND PRODYNORPHIN GENE IN THE MOUSE BRAIN

Górska Anna Maria, Kaminska Katarzyna, Wawrzczak--Bargiela Agnieszka, Przewlocki Ryszard, Golembiowska Krystyna

Institute of Pharmacology, Departments of Pharmacology and Molecular Neuropharmacology, Polish Academy of Sciences ~ Kraków ~ Poland

MDMA [(±)-3,4-methylenedioxymethamphetamine] is a psychoactive recreational drug of abuse and is often accompanied with caffeine in “ecstasy” tablets to gain stronger stimulatory effect. MDMA is known to inhibit DA and 5-HT transporters with different rank order of potency dependent of the studied species. Neurotoxic effects of MDMA were evidenced in several animal models. In mice, MDMA induces rather dopaminergic neurotoxicity, in contrast to 5-HT neurotoxicity in rats or non-human primates. It was reported that caffeine (CAF), a weak A1/A2A adenosine receptor antagonist, increases micro- and astroglial cells activation in mice treated sub-chronically with both drugs and potentiates MDMA-induced DA and 5-HT release in mouse striatum, after acute administration. This suggests that CAF may increase neurotoxic effect of MDMA.

To clarify the mechanism of neurotoxicity produced by MDMA and CAF we investigated the effect of repeated doses of both substances on DA and 5-HT release in the mouse striatum as well as their effect on the tissue content in various regions of the mouse brain. In addition mRNA expression levels of the gene encoding prodynorphin gene was tested.

Mice were treated with four doses of MDMA (10 mg/kg) given every 2 hrs and CAF (5 mg/kg) injected twice every 4 hrs. Release of DA and 5-HT was measured using microdialysis in striatum of freely moving mice. The level of DA, 5-HT and their metabolites was assayed by HPLC with coulochemical detection. The expression of prodynorphin (Pdyn) gene was analyzed using Assay-On-Demand TaqMan probes for Pdyn using the Real Time PCR System (BioRad).

MDMA increased striatal release of DA more robustly than 5-HT. CAF potentiated this effect exclusively on DA. MDMA decreased DA turnover in striatum and frontal cortex as well as 5-HT turnover in striatum, frontal cortex and hippocampus. This effect was potentiated by CAF as well. The mRNA expression level of the gene encoding opioid peptide PDYN was increased about 2,5 fold in the striatum after MDMA. CAF did not influence the expression of PDYN.

The changes observed after repeated administration of MDMA seem to result from inhibition of DA and 5-HT transporters, which leads to modification of DA and 5-HT metabolism. Potentiation of this effect by CAF indicates synergistic interaction of both psychostimulants. Increase in the mRNA expression of the gene encoding PDYN by MDMA indicates an increase in activity of strio-nigral GABA-ergic neurons via stimulation of postsynaptic D1 receptor.

P 42/09

SYSTEMIC AND LOCAL EFFECTS OF ANTIDEPRESSANTS AND KETAMINE ON CATECHOLAMINE TRANSMISSION IN THE BED NUCLEUS OF STRIA TERMINALIS: A MICRODIALYSIS STUDY IN FREELY MOVING RATS

Cadeddu Roberto, Jadzic Dragana, Carboni Ezio

Dept. of Biomedical Sciences ~ Cagliari ~ Italy

Antidepressants include a relatively wide spectrum of drugs that increase monoamines extracellular concentration (output) in many brain areas. Among them, the Bed Nucleus of Stria Terminalis (BNST) has received considerable attention because its role in modulating the stress response, but also in the acquisition and expression of emotions. Because this role, and because it is richly innervated by monoamines, BNST may be involved in aetiology of depression and in the mechanism of action of antidepressants. We recently showed that various antidepressants, independently from their mechanism of action, share the property of increasing dose dependently catecholamine transmission in the rat BNST. During the last decade, the non selective N-methyl-D-aspartate (NMDA) receptor antagonist ketamine has been shown to evoke long-lasting antidepressant effect already after infusion of a single dose. Thus, it was suggested that ketamine could be a promising medication for patients who do not respond to treatment with monoamine reuptake blockers antidepressants.

For further investigating the role of BNST in depression, we studied the effect of acute administration of ketamine on catecholamine transmission. Additionally, we investigated the local effect of ketamine in the BNST by administering through the dialysis perfusion Ringer. We also investigated the effect of the local administration of typical antidepressants that block norepinephrine and/or serotonin transporters (NET and SERT, respectively).

Dopamine (DA) and norepinephrine (NE) were assessed in dialysate samples by HPLC and coulometric detection (ESA). Samples were collected every 20 minutes (flow 1µL/min) in freely moving rats implanted in the BNST (coordinates: Antero-Posterior: -0.40; Lateral: -1.2; Vertical: -8).

(i) systemic administration of various typical antidepressants and ketamine, dose dependently, modify DA and NE output in the BNST; (ii) local perfusion of desipramine, but not citalopram, increased DA and NE output in the BNST; (iii) ketamine local perfusion in the BNST increased DA, but not NE output.

These results suggest that catecholamine transmission in the BNST may be part of a common downstream pathway that is involved in the mechanism of action of antidepressants and ketamine, and that a dysfunction of neuronal transmission in BNST may have a role in the aetiology of affective disorders.

P 43/09

FUNCTIONALIZED POLYMERIC NANOPARTICLES AS KEY TO OPEN BRAIN DOORS

Portioli Corinne[1], Bovi Michele[2], Donini Marta[3], Benati Donatella[1], Perduca Massimiliano[2], Dusi Stefano[3], Monaco Hugo[2], Bentivoglio Marina[1]

[1]Dept of Neurological and Movement Sciences, University of Verona ~ Verona ~ Italy, [2]Dept of Biotechnology, University of Verona ~ Verona ~ Italy, [3]Dept of Pathology and Diagnostics, University of Verona ~ Verona ~ Italy

The blood-brain barrier (BBB) notably exerts a strict control of molecule and cell entry into the brain for homeostatic regulation and protection. This, however, represents a severe limitation for the administration of drugs with a potential therapeutic application for the treatment of neurological diseases. Nanotechnological approaches could overcome such limitations. In particular, a promising approach employed for BBB crossing is the binding of specific ligands on the nanoparticle (NP) surface, taking advantage of receptor-mediated transport to drive the system into the brain.

The present study was aimed at assessing at short-term the BBB crossing of functionalized biocompatible and biodegradable NPs made of poly (D,L-lactide-co-glycolide) polymer (PLGA), and at testing their potential to elicit a peripheral response of immune cells and inflammatory effects in the brain parenchyma.

Custom-made PLGA NPs were functionalized with a fluorophore and conjugated with specific molecules to increase brain targeting. Targeting ligands were a peptide derived from ApoE, which mediates the lipid transport through the binding to low density lipoprotein receptor family, or the prostaglandin D synthetase protein which catalyzes the isomerization of prostaglandin PGH2 to PGD2, the major prostanoid produced in the CNS which shows a structure similar to the peptide, important for the receptor binding. The biodistribution of fluorescently-tagged and functionalized PLGA NPs was investigated with confocal microscopy and transmission electron microscopy at 2 h after peripheral administration in mice. Immunophenotyping of astrocytes and microglial cells, the main cells, which regulate the neuroinflammatory response, was pursued at 24 h. In addition, the effect of these NPs on the viability of human dendritic cells (DCs), which play a key role in the immune response, and on cytokine production was evaluated in vitro.

Following iv injections, targeted-PLGA NP localization was found mostly on neuronal and microglial cell membranes, besides the extracellular space, as also confirmed by ultrastructural analysis. No signs of neuronal cell death were found up to 24h from administration. A mild astrocyte and microglia activation was observed in the brain. No significant changes in the DCs response or cytokine production were detected in vitro.

The data point out that functionalized PLGA NPs can cross the BBB and enter the brain parenchyma. This approach combines the advantages of a biodegradable and biocompatible polymer with brain targeting peptides as potential drug delivery systems for neurological disease therapy.

The support of the "Verona Nanomedicine Initiative" is gratefully acknowledged.

P 44/09

GAD67 IMMUNOREACTIVITY IN THE CENTRAL AMYGDALA AS A MARKER OF LONG-LASTING CHANGES IN NEURONAL ACTIVITY CAUSED BY ADOLESCENT PRE-EXPOSURE TO DRUGS OF ABUSE

Sil Annesha[1], Pisanu Augusta[2], Lecca Daniele[1], Scifo Andrea[1], Cadoni Cristina[2], Di Chiara Gaetano[1]

[1]Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari ~ Cagliari ~ Italy, [2]Institute of Neuroscience, National Research Council Of Italy ~ Cagliari ~ Italy

Numerous studies suggest that early exposure to drugs of abuse can induce long-lasting neurochemical and behavioural alterations in adulthood. Lewis (LEW) and Fischer 344 (F344) rats have been widely used to study these alterations, given their different vulnerability to drug addiction. Previous studies have implicated the expression of GAD67 (glutamate acid decarboxylase-67) mRNA in the central nucleus of the amygdala (CeA) as a marker for some of these changes, i.e. behavioral sensitization. Using fluorescent immunohistochemical techniques, we tested the validity of GAD67 as a neuronal marker predictive of these changes in the aforementioned strains in the CeA.

Mid-adolescent rats underwent repeated, dose-escalated exposure (3 days) to either morphine, heroin or THC (tetrahydrocannabinol) to ascertain the presence of irreversible changes which may be carried into adulthood. Pre-exposure to morphine/heroin caused a decrease in GAD67-immunoreactivity (GAD67-IR) in F344 rats and no change in LEW rats at adulthood, while THC pre-exposure did not produce any significant changes in GAD67 in both strains.

In a second experimental setting, adolescent THC pre-exposure was followed by heroin self-administration at adulthood, followed by an extinction training and drug induced reinstatement. 3-4 days after reinstatement significant increase in GAD67-IR was observed in THC-pretreated F344 rats but not in their controls, while no change was observed in LEW strain

Since after both experimental setting F344 rats appear to be behaviorally sensitized to opiates, but not to THC, the results obtained suggest that an increased GAD67 expression cannot be considered a mere marker of behavioral sensitization since both a decrease or an increase was observed after two different experimental settings.

On the other hand LEW rats did not show either behavioral sensitization to opiates or any significant change of GAD67 expression in spite of long-lasting changes in reward system and emotional function. Given the different effects of the above treatments in the two strains, not univocally predictive of long-lasting changes in adulthood, we suggest that different changes of GAD67 in F344 and LEW rats might be due to opposite responsivity of HPA system in the two strains (hyper in F344 and hypo in LEW). Since the strain more affected by THC pre-exposure, in terms of changes of reward and emotional function, is the LEW strain, it might be hypothesized that the increase in GAD67 observed in F344 strain is a contra adaptive mechanism contrasting progression toward drug addiction and thus not correlated to the individual vulnerability to drug addiction but rather a reaction to an aversive state as withdrawal.

P 45/09

IMMUNOCHEMICAL LOCALIZATION OF GABAA RECEPTOR SUBUNITS IN THE FRESHWATER POLYP HYDRA VULGARIS (CNIDARIA, HYDROZOA)

Locci Andrea[1], Imperatore Roberta[2], Santoru Francesca[1], Porcu Patrizia[3], Cristino Luigia[2], Pierobon Paola[4], Concas Alessandra[1]

[1]Department of Life and Environment Sciences, University of Cagliari, Cagliari, Italy, [2]Institute of Biomolecular Chemistry, CNR, Pozzuoli, Italy, [3]Neuroscience Institute, National Research Council of Italy (CNR), Cagliari, Italy, [4]Institute of Applied Sciences and Intelligent Systems E. Caianiello, C.N.R., Pozzuoli, Italy

γ-aminobutyric acid (GABA) is the most widely distributed inhibitory neurotransmitter in both vertebrate and invertebrate species. GABA receptors have been described in several invertebrate species of different phyla. Despite the considerable variability of results, it is becoming clear that in many invertebrate species GABA receptors present a pharmacology comparable to that of mammalian ones without fitting precisely into the classification developed for mammalian brain. We have demonstrated that GABA is present in Hydra tissues and that Hydra responds to GABA positive allosteric modulators.

To approach the molecular characterization of GABA receptors, we examined the occurrence and distribution of GABAA receptor subunits in Hydra polyps.

The presence and localization of GABAA receptor subunits in Hydra was examined by Western Blot in membrane preparations and immunohistochemical analysis of whole mount preparations. The presence of GABAA receptor subunits in Hydra membrane preparations, was tested using different antibodies against α1, α2, α3, β1, β2, β3, γ1, γ2, γ3, δ, p2□and ε subunits, each probed with the corresponding peptide. In membranes incubated with the α3 antibody the unique band inhibited by the specific peptide appeared at 35 kDa. Protein bands blotted with the β1 antibody occurred at 60 kDa, while those blotted with γ3 and δ antibodies revealed bands at approximately 50 kDa and 52 kDa, respectively. None of the other subunit antibodies examined revealed protein bands in Hydra membranes. Immunohistochemical analysis by α3 and β1 antibodies revealed a diffuse immunoreactivity in tentacles, hypostome, and upper part of the gastric region. In the peduncle, double labelling clearly showed co-localization of α3 and β1 immunoreactivity on a circular structure above the foot; in the tentacles, occasional cell bodies also exhibited double staining in the form of patches. By contrast both γ3 and δ antibodies obtained a strong labelling in the lower gastric region and in the peduncle; occasional immunoreactive cells were observed in the hypostome and tentacles. Double labelling showed co-localization of α3/γ3 immunoreactivity in granules or cells in the gastric region, and in the peduncle, where circular fibers above the foot were clearly double-stained. Finally, co-localization of α3 and δ immunoreactivity was observed in cells of the tentacles, the gastric region and the peduncle.

These data indicate that populations of specific subunits of the GABAA receptor are present in Hydra vulgaris, the most primitive metazoans to have developed a nervous system.

P 46/09

SELECTIVE ACTIVATION OF PARVALBUMIN- OR SOMATOSTATIN-EXPRESSING INTERNEURONS TRIGGERS EPILEPTIC SEIZURELIKE ACTIVITY IN MOUSE MEDIAL ENTORHINAL CORTEX

Yekhlef Latefa, Breschi Gianluca, Lagostena Laura, Russo Giovanni, Taverna Stefano

Istituto Italiano Di Tecnologia ~ Genoa ~ Italy

GABAergic interneurons are thought to play a critical role in eliciting interictal spikes (IICs) and triggering ictal discharges in temporal lobe epilepsy, yet the contribution of different interneuronal subtypes to seizure initiation is still largely unknown.

A selective manipulation of parvalbumin (PV) or somatostatin (SOM)interneurons may play a key role in the ictogenesis process.

Here we took advantage of optogenetic techniques combined with patch-clamp and field recordings to selectively stimulate parvalbumin PV- or SOM- positive interneurons expressing channelrhodopsin-2 (CHR-2) in layers II-III of adult mouse medial entorhinal cortical slices during extracellular perfusion with the proconvulsive compound 4-aminopyridine (4-AP).

In control conditions, blue laser photostimulation selectively activated action potential firing in either PV or SOM interneurons and, in both cases, caused a robust GABAA-receptor-mediated inhibition in pyramidal cells (PCs). During perfusion with 4-AP, brief photostimuli (300 ms) activating either PV or SOM interneurons induced patterns of epileptiform activity that closely replicated spontaneously occurring IICs and tonic-clonic ictal discharges. Laser-induced synchronous firing in both interneuronal types elicited large compound GABAergic inhibitory postsynaptic currents (IPSCs) correlating with IICs and preictal spikes. In addition, spontaneous and laser-induced epileptic events were similarly initiated in concurrence with a large increase in extracellular potassium concentration. Finally, interneuron activation was unable to stop or significantly shorten the progression of seizurelike episodes.

These results suggest that entorhinal PV and SOM interneurons are nearly equally effective in triggering interictal and ictal discharges that closely resemble human temporal lobe epileptic activity.

P 47/09

FOXG1 ANTAGONIZES CORTICO-CEREBRAL ASTROGENESIS

Grudina Clara, Falcone Carmen, Blecich Nika, Santo Manuela, Mallaci Antonello

SISSA ~ Trieste ~ Italy

Foxg1 is a transcription factor gene involved in key steps of early cortico-cerebral development, including specification of the telencephalic and cortical fields, tuning of proliferation/differentiation kinetics, radial migration of projection neurons and laminar specification of them. Its allele dosage is crucial. Hemizygosity for Foxg1 and duplication of it result into two devastating nosological entities, namely the Rett and West syndromes, respectively. We previously showed that Foxg1, like its Drosophila m. ortholog sloppy paired, also antagonizes gliogenesis.

Aim of this study was to investigate the role played by this gene in two aspects of cortico-cerebral astroglial development, namely early commitment of neural stem cells towards glial fates and subsequent implementation of the astrocytic differentiation program.

These issues were addressed in mouse and human models. In the first case, we took advantage of Foxg1-gain- and Foxg1-loss-of-function transgenic animals, in vivo electroporated brains and primary cultures of cortical precursors engineered by lentiviral vectors. As for human models, we relied on embryonic pallial precursors manipulated by lentiviral vectors and SFEBq aggregates derived from induced pluripotent stem cells.

We found that Foxg1 overexpression in pallial stem cells reduces their astroglial output, regardless of the developmental time window and the model system taken into account. An opposite effect is elicited by halving Foxg1 gene dosage. All that is likely due to altered commitment of stem cells to astroglial fates. Moreover Foxg1 overexpression also interferes with selected aspects of late astrocyte differentiation, possibly jeopardizing anti-excitotoxic capability of these cells.

These findings may help to reconstruct the molecular logic underlying normal articulation of astrogenesis. Moreover they provide useful hints about pathogenetic mechanisms leading to neurological disorders triggered by altered Foxg1 dosage.

P 48/09

METABOLIC FATE AND EFFLUX OF CHOLESTEROL IN THE HIPPOCAMPUS OF DIABETIC MALE RAT

Romano Simone, Giatti Silvia, Pesaresi Marzia, Mitro Nico, Cermenati Gaia, Caruso Donatella, Melcangi Roberto Cosimo

Department of Pharmacological and Biomolecular Sciences, Center of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano - Via Balzaretti 9 ~ Milano ~ Italy

Diabetic encephalopathy (DA) is one of the complications of diabetes affecting the central nervous system (CNS). DA is associated with neurophysiological changes, cognitive deficits, increased risk of dementia and psychiatric disorders. The molecular mechanisms underlying the DA are still poorly understood. Interestingly, preliminary experimental observations have shown that diabetes alters brain cholesterol metabolism.
Brain is one of the most cholesterol-rich organs, since almost all brain cholesterol is derived by de novo synthesis. Cholesterol plays an important role in myelin synthesis and it has been implicated in regulation of many biochemical processes. Recent observations indicate that in neurodegenerative and psychiatric disorders the synthesis and/or metabolism of cholesterol as well as of key physiological regulators of nervous function, such as neuroactive steroids (i.e., molecules derived from cholesterol) are affected.

To verify, in an experimental model of type 1 diabetes (i.e. streptozotocin-diabetic rats), the possible alterations of metabolic fate and efflux of cholesterol at the level of hippocampus.

Diabetes was induced in Sprague Dawley male rats by a single intraperitoneal injection of streptozotocin. After 1 month of diabetes the total content of cholesterol, desmosterol, 24-hydroxy cholesterol as well as the levels of twelve different neuroactive steroids were analyzed by liquid chromatography tandem mass spectrometry assay (LC-MS/MS) in the hippocampus. qPCR was used to determine the expression of genes involved in metabolism and efflux of cholesterol and steroidogenesis.

Data obtained by LC-MS/MS, show altered levels of a cholesterol precursor, such as desmosterol, and metabolites (e.g., 24-hydroxy cholesterol). In agreement, gene expression of cholesterol 24-hydroxylase, metabolizing cholesterol into 24-hydroxy cholesterol, was up regulated by diabetes. Moreover, ATP-Binding cassette (ABC)A1 and ABCG1 genes, responsible of the efflux of cholesterol metabolites, were also up-regulated. Furthermore the levels of neuroactive steroids, like for instance pregnenolone (PREG), testosterone (T) and its metabolite dihydrotestosterone (DHT) were decreased. In agreement, mRNA levels of a molecule (i.e., StAR) involved in the transport of cholesterol into mitochondria, where PREG is synthesized, and of 5alpha-reductase (i.e., enzyme converting T in DHT) were down-regulated.

Results here reported indicate, for the first time, that in the hippocampus of diabetic male rats the metabolism of cholesterol into 24-hydroxy cholesterol, efflux, as well as conversion of cholesterol into neuroactive steroids were affected. These observations may represent a molecular basis for cerebral complications observed in DA.

The financial support of Fondazione Cassa di Risparmio delle province Lombarde (CARIPLO) to R.C. Melcangi is gratefully acknowledged

P 49/09

EFFECTS OF LEPTIN ON MOUSE CHROMAFFIN CELLS FIRING AND CATHECOLAMINES SECRETION

Gavello Daniela, Vandael David, Gosso Sara, Carbone Emilio, Carabelli Valentina

University of Torino ~ Torino ~ Italy

Leptin is an adipokine produced by the adipose tissue regulating body weight through its appetite-suppressing effect. Besides being expressed in the hypothalamus and hippocampus, leptin receptors (ObRs) are also present in chromaffin cells of the adrenal medulla, although little is known about its signalling pathway and regulation.

Here we report the effect of leptin on mouse chromaffin cells. The aim is to understand the effect of this adipokine on cell excitability and catecholamines secretion.

To this purpose we performed current-clamp, voltage-clamp and single-channel recordings on primary cultures of mouse chromaffin cells (MCCs) (Vandael et al J. Physiol. 593:905, 2015). Moreover we studied catecholamine secretion by combining capacitance and amperometric recordings (Carabelli et al., J. Physiol. 581:149, 2007).

Acute application of leptin (1 nM) on spontaneously firing MCCs caused a slowly developing membrane hyperpolarization followed by complete block of action potentials (APs) firing. This inhibitory effect at rest was abolished by the BK channel blocker paxilline (1 μM), suggesting the involvement of BK potassium channels. Single-channel recordings in “perforated microvesicles” confirmed that leptin increased BK channel open probability without altering its unitary conductance. BK channel up-regulation was associated to the PI3K signaling cascade, as the PI3K specific inhibitor wortmannin (100 nM) fully prevented BK current increase.
We also tested the effect of leptin on evoked AP firing and Ca2+-driven exocytosis. While leptin preserves well-adapted AP trains of lower frequency, APs are broader and depolarization-evoked exocytosis is increased due to the larger size of the ready-releasable pool and higher frequency of vesicle release. Kinetics and quantal size of single secretory events remained unaltered. Leptin had no effects on firing and secretion in db–/db– mice lacking the ObR gene, proving its specificity.

Our data suggest that leptin exhibits a dual action on MCCs activity. It dampens AP firing at rest but preserves AP firings and increases catecholamine secretion during sustained stimulation, highlighting the importance of the adipo-adrenal axis in the leptin-mediated increase of sympathetic tone and catecholamine release.

P 50/09

T-TYPE CALCIUM CHANNELS IN MOUSE CHROMAFFIN CELLS: CHARACTERIZATION AND SENSITIVITY TO STRESSFUL STIMULI

Calorio Chiara, Marcantoni Andrea, Carbone Emilio

Department of Drug Science, University of Turin ~ Turin ~ Italy

The presence of T-type calcium channels (TTCCs) in mouse chromaffin cells (MCCs) has been recently highlighted but a detailed characterization is still missing. As T-type channels are shown to control the unusual “low-threshold” neurosecretion of neuroendocrine cells and central neurons we decided to investigate the degree of expression and gating properties of MCCs T-type channels.

The aims of this work are: 1) study the expression and characterization of TTCCs in MCCs, 2) elucidate their role in “low-threshold” secretion and 3) investigate their sensitivity to stressful stimuli (chronic hypoxia, high glucose, corticosterone application)

All experiments were performed in current-clamp and voltage clamp recording conditions on primary culture of mouse chromaffin cells(MCCs). The secretory response was studied by membrane capacitance changes.

Our results show that:
1. In [Ca2+]=10mM 68% of MCCs exhibits T-type calcium currents (Vh = -90 mV) in I/V ramps or square pulse depolarizations, whose identity was confirmed by:
a. their availability at potentials below -30 mV
b. fast and complete inactivation at voltages where they are fully activated (time constant of inactivation 18.5 ms at -30 mV)
c. slow deactivation on return to -50mV after brief depolarizations at -30 mV to reach maximal TTCC activation
d. strong block by TTA-P2 (Cav3 channels specific blocker)
2. Stressful stimuli like chronic hypoxia (CH, 3% of O2 for 15-18h) increase to 79% the MCCs expressing TTCCs. Moreover this effect is mimicked by the iron-chelating agent deferoxamine mesylate salt (DFX, 300 µM), which activates HIF1α and leads to 75% the MCCs expressing TTCCs.
3. The "low-threshold" exocytosis in MCCs is mainly carried by TTCCs since the cells expressing these channels exhibited an overall exocytosis and quantity of Ca2+ charge more prominent between -50 mV and -20mV when compared to the hypoxic cells not expressing TTCCs.

To our knowledge, this is the first report identifying TTCCs in MCCs, openings the possibility of further tests on the role of these channels in the genesis of pathologies associated with adrenal chromaffin cells, like chronic stress and hypertension, using KI and KO mouse models.

P 51/09

AN INTEGRATED APPROACH FOR MORPHOFUNCTIONAL ANALYSIS OF DRGS IN NORMAL AND DIABETIC MICE

Ciglieri Elisa[2], Ferrini Francesco[2], Tonti Simone[1], Di Cataldo Santa[1], Ficarra Elisa[1], Salio Chiara[2]

[1]Dipartimento di Automatica e Informatica, Politecnico di Torino ~ Torino ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Torino ~ Torino ~ Italy

Nociceptive sensory neurons in dorsal root ganglia (DRGs) are first-order neurons conveying pain information to higher centers. Physiological pain has a protective role, which is disrupted in several pathologies leading to abnormal inflammatory or neuropathic pain. Diabetic polyneuropathy (DPN) is a common complication of diabetes and affects up to fifty percent of diabetic patients, some of which display neuropathic pain.

To develop an integrated method for investigating the neurochemical and functional properties of DRG neurons in normal and diabetic mice; to analyze the spatial relationships of DRG neurons in a whole-mount preparations.

CD1 male mice were made diabetic after a single intraperitoneal injection of streptozotocin (150 mg/Kg) at P30, while controls received vehicle only. Glucose levels and nociceptive behaviour were weekly monitored. All animals were sacrificed at P60. DRGs were acutely excised and the connective tissue was dissolved by incubation in 5-10 mg/mL collagenase. The entire DRGs were then used for patch-clamp recordings and, subsequently, for immunofluorescence, as the elimination of the connective capsule facilitated the access of the recording pipette as well as the penetration of primary antibodies. DRGs were stained for two classical phenotypic markers of nociceptors, i.e. the calcitonin gene-related peptide (CGRP) and the isolectin B4 (IB4) by a rabbit antibody and a biotin-conjugate, respectively. Whole DRGs Z Stacks were then collected using confocal microscopy. 3D quantitative analysis was performed using home-developed software, which provided automated counting of the immune-labeled neurons and quantification of their 3D morphological characteristics.

Pre-treatment with collagenase was found to improve reagent penetration in our whole-mount preparations, allowing an easy identification of the main populations of nociceptors (CGRP+ peptidergic and IB4+ non-peptidergic). The phenotypic identification of recorded neurons was possible thanks to the injection of a fluorescent tracer in the recorded neurons. Functional analysis confirmed an increase of pain hypersensitivity in diabetic mice which was paralleled by a decreased firing latency in DRG neurons. This difference was prominent in small cells (<25 µm). Consistent with functional analysis, 3D quantitative immunofluorescence revealed significant changes of CGRP and IBP-labeled structures between control and diabetic mice that can be related to DPN.

This method is a flexible in vitro approach that preserves the neuroanatomical relationships between individual neurons, allowing also a functional analysis of their electrophysiological

properties. The application of such a method in DRGs obtained from diabetic mice will shed new light on the morphofunctional alterations occurring in DPN.

P 52/09

TARGETING SUBSTANTIA NIGRA THROUGH OXYTOCIN RECEPTORS

Angioni Laura[1], Cocco Cristina[1], Argiolas Antonio[2], Ferri Gian Luca[1], Melis Maria Rosaria[2], Sanna Fabrizio[2]

[1]NEF-Laboratory, Department of Biomedical Science, University of Cagliari ~ Cagliari ~ Italy, [2]Neuropsychobiology Laboratory, Department of Biomedical Science, University of Cagliari ~ Cagliari ~ Italy

Recent studies suggest a role of oxytocin in the control of locomotor activity. Interestingly, studies in the brain of rats and humans have shown that oxytocinergic nerve endings and receptors are present in the substantia nigra (SN), a brain area of basal ganglia circuitry. Accordingly, a loss of nigrostriatal dopaminergic neurons causes Parkinson disease, characterized by severe motor disturbances including hypomotility.

In order to prove a functional oxytocin-dopamine interaction in the SN, the effect of the injection into the SN of the novel cytotoxin Oxytocin-Saporin (Oxy-SAP), which selectively destroys cells expressing oxytocin receptors, was studied in male rats.

Male Sprague Dawley rats (n=24) were monolaterally or bilaterally injected into the SN with Oxy-SAP or vehicle (PBS). Locomotor activity was assessed before and two and four weeks after injection (Digiscan Animal Activity Analyser). Immunohistochemistry was used to verify the presence and extent of the lesion in the dopaminergic neurons and to investigate any modifications in the GABAergic and glutamatergic systems.

After four weeks, animals bilaterally injected with Oxy-SAP showed a significant increase in horizontal locomotor activity (50-60%) and in vertical locomotor activity as well, when compared to PBS-injected rats.
In Oxy-SAP injected animals, Tyrosine Hydroxylase (TH) immunoreactivity (-ir) was reduced in SN compacta somas and dendrites coursing into the pars reticulata. TH-ir decrease was more pronounced in the areas of the SN adjacent to the injection site and less, although still present, in those further away from it. No evident variation in Glutamate Decarboxylase-ir was observed. Interestingly, preliminary results showed a reduction in SN vesicular glutamate transporters (VGluT1, VGluT2 and VGluT3)-ir, possibly correlated with the dopaminergic lesion extent, suggesting the potential induction of alterations also in the glutamatergic system. In particular, VGluT1-ir reduction was almost complete in SN reticulata and slightly less in the compacta, while VGluT2- and VGluT3-ir were reduced in both compacta and reticulata, with VGlut2 being the most affected. In line with these results, an increase in locomotor activity was also observed in rats with a loss of the nigrostriatal dopaminergic neurons induced by the neurotoxin 6-hydroxydopamine, when antagonists of glutamatergic receptors were injected into the SN.

In conclusion, oxytocin receptors in the SN seem to be involved in the control of dopaminergic and glutamatergic neurons but not of GABAergic neurons. Moreover, these results confirm the importance of the modulation of nigral glutamatergic transmission as a possible treatment of basal ganglia movement disorders.

P 53/09

EFFECTS OF PRENATAL STRESS EXPOSURE AND MATERNAL SEPARATION ON HPA AXIS RESPONSIVENESS AND BEHAVIOR IN YOUNG ADULT MALE RATS

Biggio Francesca[1], Locci Valentina[3], Boero Giorgia[1], Pisu Maria Giuseppina[2], Olla Pierluigi[1], Serra Mariangela[1]

[1]Department of Life and Environmental Sciences ~ University of Cagliari ~ Italy, [2]Institute of Neuroscience, National Research Council ~ University of Cagliari ~ Italy, [3]Department of Biomedical Sciences ~ University of Sassari ~ Italy

It is known that a negative association between number of early adverse experiences and age could may fall in long-term effects on developement and emotional/behavioral states inducing deep, and sometimes irreversible changes in the adulthood. Therefore vulnerability to psychopathologies in the adult can be predicted from the prenatal as well as postnatal experiences.

We studied whether a moderate dose of ethanol (1g/kg,), during the pregnancy (GD17-20) and stress induced by daily maternal separation (MS 3h, PDN 3-15) alters emotional behavior and sensitivity to acute stress in adult offspring. Because maternal separation stimulates HPA activity of the dams and changes of corticosterone levels influence maternal care, we evaluated if exposure to ethanol during pregnancy may influence maternal behaviour. The responsiveness of the HPA axis to stressful conditions was evaluated in young adult male rats by measuring the basal and foot shock-stimulated plasma levels of corticosterone as well as allopregnanolone.

Starting from GD17 throughout GD 20 Sprague-Dawley dams from EtOH group will be daily administered 1 g EtOH/kg. At PND 3 throughout PND15, litters from each group (VEH and EtOH) were subjected to maternal separation (3h). For the experimenti we used RIA and ELISA to steroids measurements, Foot-shock stress to measure HPA responsiveness, elevated-plus-maze to evaluate the anxiety state and maternal care.

We found that prenatal ethanol exposure and subsequent maternal separation (EtOH-MS group) resulted in a decrease in plasmatic corticosterone and allopregnanolone levels compared to counterpart not subjected to maternal separation (EtOH-NMS). Furthermore, the enhancement of corticosterone and allopregnanolone levels induced by foot-shock stress in EtOH-MS was remarkably increased in comparison with animals that were just exposed to prenatal ethanol. Besides, elevated plus maze test shows an increase in anxious behavior in EtOH-MS group compared to counterpart (EtOH-NMS). We discovered that maternal separation induced an increase in arched-back nursing and pup-licking in mothers not exposed to ethanol. Furthermore, it is interesting that the maternal care of dams separated from their pups are not affected by exposure to ethanol during the last days of pregnancy.

This result suggests that changes in emotional state and stress response in animals subjected to prenatal stress and subsequent maternal separation is not influenced by the quality of maternal care received. In conclusion, stressful events that occur during pregnancy and childhood may alter the responsiveness of HPA axis in adulthood.

P 54/09

SOCIAL ISOLATION INDUCES A DISREGULATION OF HPA AXIS ACTIVITY IN MALE RATS

Pisu Maria Giuseppina[1], Boero Giorgia[2], Olla Pierluigi[2], Biggio Francesca[2], Serra Mariangela[2]

[1]Institute of Neuroscience of the National Research Council ~ Cagliari ~ Italy, [2]Department of Environmental and Life Sciences, Section of Neuroscience and Anthropology, University of Cagliari ~ Cagliari ~ Italy

Social isolation from weaning induces different behavioral and neurochemical alterations in comparison with group-housed age-matched controls, suggesting that socially isolated rodents represent an animal model of neuropsychiatric disorder. Social isolation results, therefore, in a decrease in the brain and plasma concentrations of neuroactive steroids that act as positive modulators at GABAA receptors and is accompanied by a hyperesponsiveness of HPA axis to acute stressful stimuli. It also increases the sensitivity of the pituitary to corticotropin-releasing factor (CRF) and alters HPA axis negative feedback regulation.

The aim of this research was to study mechanisms that underlie the altered HPA reactivity to acute stress. For this purpose we measured plasma corticosterone, hypothalamic CRF and the expression of hippocampal and hypothalamic glucocorticoid receptors (GR) at different time point after acute stress (foot-shock, 5 min) exposure.

The enzyme immunoassay was used for the quantitative determination of plasma corticosterone and performed according to the manufacturer's instructions (corticosterone ELISA, IBL International, Germany). Immunoblot analyses were performed with polyclonal antibodies to CRF (1:200, SC10718), GR (1:200, SC1002) and glyceraldehyde-3-phosphate dehydrogenase (1:5000, MAB374).

Corticosterone levels were significantly increased in group-housed animals after 5 min, reached a peak at 15 min and returned to baseline 60 min after stress. At variance, plasma corticosterone levels of socially isolated rats were increased at 5 min after stress, were maximum at 60 min and remained significantly high 7 hours later. In the hypothalamus of group-housed animals no changes in the levels of CRF were detected in any time point measured, while in socially isolated rats a significant increase of CRF levels was found at 15 min, 30 and 60 min after the end of the stress. Thus, these data demonstrate that social isolation results in hypothalamic hyperactivity following acute stress exposure and suggest that socially isolated rats are much less sensitive to glucocorticoid negative feedback than group-housed animals. Social isolation also increased basal levels of both hippocampal and hypothalamic membrane GR. Moreover, in group-housed rats, GR expression progressively increased with the time after foot-shock exposure, becoming statistically significant 90 min after stress, whereas socially isolated rats did not show any change in hippocampal and hypothalamic GR expression at any time point examined.

These results suggest that the altered GR expression induced by social isolation stress during adolescence may underlie the modifications in the activation of HPA axis and in negative feedback, thus resulting in a prolonged response to acute stress.

P 55/09

ALTERED MATERNAL STYLE IN A MOUSE MODEL OF IDIOPATHIC AUTISM: A ROLE FOR OXYTOCIN?

Giorgi Maria Carla[1], Cutuli Debora[2], De Felice Alessia[1], Farioli Vecchioli Stefano[2], Calamandrei Gemma[1]

[1]Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy ~ Roma ~ Italy, [2]Institute of Cell Biology and Neurobiology, National Research Council, Fondazione S.Lucia ~ Roma ~ Italy

Different parental styles can permanently affect DNA methylation, gene expression and neural functions in the offspring, influencing long-term behavior and development of various diseases through epigenetic mechanisms. In such framework, deficits in maternal care and bonding may be an important contributing factor in the development of anxiety and depression and other behavioural disturbances in the offspring. The contribution of the maternal style has been scarcely investigated so far in murine models of neurodevelopmental disorders, although it is likely that altered maternal behaviour could contribute to the abnormal behavioural phenotype of the offspring.

We performed a thorough characterization of the maternal care repertoire of BTBR mice, an inbred strain which display disturbances in social responses and represent a validated model for the study of autism spectrum disorders (ASD).

Lactating female mice of the BTBR and C57BL/6J strain were analyzed at different time of the day during the two first weeks postpartum as for nest building activity, maternal behavior (licking, crouching, retrieving) and time spent in or out of the nest. Nest defense and aggression towards an intruder male followed by retrieving test was also performed on post partum day 7. Expression levels of mRNA and protein of selected neuroendocrine markers (oxytocin and oxytocin receptor) were analysed in a separate group of adult BTBR females by PCR and immunohistochemical staining in brain areas implicated in maternal behaviour.

Results demonstrate significant alterations in the maternal style of BTBR mice. BTBR females spent less time in the nest in the first week post partum in comparison to C57 females. On post partum day 2, BTBR dams exhibited more pup sniffing, and lower levels of digging in comparison to controls. As for maternal aggression frequency and latency of attack behavior towards the intruder male were similar in both strains; however, BTBR dams showed elevated levels of repetitive digging and grooming associated with low levels of nest defense. BTBR females showed a significant increase of mRNA expression of oxytocin in the hypothalamus in comparison with C57 females; at variance, preliminary immunohistochemistry data suggest lower oxytocin levels in specific hypothalamic nuclei of BTBR females.

Our findings suggest that the autistic-like phenotype of the BTBR mice influences their mothering style. The well known role of oxytocin in both social and maternal responsiveness suggests that this neuropeptide might be mechanistically implicated in the behavioural phenotype of this ASD mouse model.

P 56/09

CXCL-10 AND SLEEP CHANGES AS BRAIN DISEASE BIOMARKERS IN EXPERIMENTAL SLEEPING SICKNESS

Tesoriero Chiara[2], Laperchia Claudia[2], Colavito Valeria[2], La Verde Valentina[1], Grassi-Zucconi Gigliola[2], Rogers Jean[3], Kennedy Peter Ge[4], Montague Paul[4], Bentivoglio Marina[2]

[1]Dept. of Biotechnology, University of Verona ~ Verona ~ Italy, [2]Dept. of Neurological and Movement Sciences, University of Verona ~ Verona ~ Italy, [3]Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow ~ Glasgow ~ United Kingdom, [4]Institute of Infection, Immunity and Inflammation, University of Glasgow ~ Glasgow ~ United Kingdom

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease caused by subspecies of the extracellular protozoan parasite Trypanosoma brucei (T.b.). HAT is a complex neuropsychiatric syndrome, characterized by disruption of sleep-wake alternation and sleep structure changes. The disease, which is fatal if left untreated, evolves from an early hemolymphatic stage (S1) to a meningoencephalitic stage (S2) due to T.b. invasion of the central nervous system. Disease staging is currently based on the presence of parasites and counting of white blood cells in the cerebrospinal fluid (CSF) but such criteria lack sensitivity. The identification of new tools for HAT staging is, therefore, a critical need, since S1 and S2 patients are treated with different drugs and S2 therapy is very toxic.

The present study was aimed at identifying S2 potential biomarkers in experimental sleeping sickness. In particular, we focused on the chemokine CXCL-10, previously found to play a key pathogenetic role in parasite neuroinvasion.

The study was performed in T.b. brucei-infected adult rats at 6, 14, 21, 30 days post-infection (dpi) vs matched saline-treated, control animals. Notably, it was ascertained in previous studies that T.b. brucei neuroinvasion initiates at 11-14 dpi. The levels of the interferon-γ–dependent chemokines cxcl-9, cxcl-10, cxcl-11 transcripts were measured by qRT-PCR in the brain at 6, 14 and 21 dpi. The level of CXCL-10 was analysed by ELISA in both the serum and CSF at 6, 14, 21 and 30 dpi. Polysomnographic study of sleep-wake parameters, obtained by telemetric recordings, was pursued at the same time points.

The expression levels of cxcl-9, cxcl-10, cxcl-11 mRNAs progressively increased in the brain at 14 dpi and persisted very high at 21 dpi (4.8 fold increase vs uninfected controls). Interestingly, CXCL-10 levels showed a steep increase in the serum at 14 dpi (43.61 $\pm$ 17.96 pg/nmol) and later, at 21 dpi, in the CSF (113.03 $\pm$ 26.12 pg/nmol). Electroencephalographic findings indicated progressive sleep-wake fragmentation and invasion of wakefulness time by sleep.

Our findings indicate that the chemokine CXCL-10 level in the CSF could represent a biomarker for HAT S2 in correlation with sleep-wake changes, which can be easily documented in humans by actigraphy.

P 57/09

REPEATED CYCLES OF CHRONIC INTERMITTENT ETHANOL EXPOSURE ALTER GABAA RECEPTOR GENE EXPRESSION

Floris Gabriele[1], Asuni Gino Paolo[1], Mercante Beniamina[2], Deriu Franca[2], Follesa Paolo[1]

[1]University of Cagliari ~ Cagliari ~ Italy, [2]University of Sassari ~ Sassari ~ Italy

Ethanol exposure produces alterations in the gene expression of GABAA receptors (GABAAR) in the mouse brain.

Our goal is to examine the relationship between voluntary ethanol consumption and GABAAR gene expression in the hippocampus of ethanol dependent and non-dependent C57BL/6J mice by using repeated cycles of chronic intermittent ethanol exposure (CIE), known to increase voluntary ethanol intake.

Adult male mice subjected to 1 to 4 cycles of CIE in inhalation chambers (16h/day/week) were compared with matching control mice that inhale air. Each cycle of ethanol exposure was alternated with weekly limited access two-bottle choice ethanol (15%) and water consumption (2hr/day, 5 days/week).

In agreement with previous work we confirmed that CIE mice showed statistically significant increases in voluntary ethanol consumption compared to control mice. This effect was already evident starting from the first cycle to become statistically significant at the second cycle until the fourth cycle. Mice were sacrificed at different time point: end of the fourth cycle of inhalation session; end of the forth two-bottle choice session; after 8 hours withdrawal of the fourth cycle of inhalation. The hippocampus was dissected and used for mRNA extraction and RT-PCR measurement of the different GABAAR subunits. Our results showed that the expression of both delta and alpha4 subunits of the GABAAR in CIE mice, compared to control mice, were not statistically different when measured at the end of the last two-bottle session of the fourth cycle. Nevertheless, within the CIE experimental groups tested at the three different time points the expression levels of the delta subunit was statistically different while the alpha4 remain unchanged. The abundance of delta subunit were increased at the end of the fourth cycle of forced intoxication by inhalation, compared to the end of the fourth two-bottle free drinking session. By contrast, at 8 hours of withdrawal after last inhalation the delta subunit was decreased with respect of the other two experimental groups. BEC measurements in the three experimental groups revealed that the expression of delta subunit of the GABAAR fluctuate depending on ethanol intoxication state.

These preliminary findings suggest that the ethanol sensitive delta subunit of the GABAAR in the hippocampus of ethanol dependent mice may adapt its expression in response to ethanol-mediated disruption of the GABAergic neurotransmission.

P 58/09

VOLUNTARY ETHANOL DRINKING IN GABAB KNOCK-OUT MICE: EFFECT OF GAMMA-HYDROXYBUTYRIC ACID

Floris Gabriele[1], Asuni Gino Paolo[1], Mercante Beniamina[2], Deriu Franca[2], Follesa Paolo[1]

[1]University of Cagliari ~ Cagliari ~ Italy, [2]University of Sassari ~ Sassari ~ Italy

Gamma-hydroxybutiric acid (GHB) reduces self-administration of alcohol in laboratory animals.

Our goal was to study the role of GABAB receptors in this action of GHB by using GABAB knock-out mice. To achieve this goal we used the original Balb/c strain of GABAB(1) knock-out mice crossed with FVB mice in our laboratory.

Male knock-out (KO), heterozygous (ET) and wild type (WT) animals were used and compared in this study. Mice were offered ethanol using the 2 hours, 2 bottles choice drinking paradigm (ethanol 15%). After establishing baseline drinking mice were tested and monitored for 4 weeks. A group of animals received an i.p. injection of GHB at the dose of 100 mg/kg 15 minutes before the ethanol drinking session.

Our results show that voluntary ethanol consumption was significantly increased, starting at the second week of treatment, in both KO and ET mice. Over 4 weeks of treatment the average amount of ethanol consumed during the 2 hour access was 1.71 g/Kg ($P<0.01$) and 1.87 g/kg ($P<0.001$) for KO and ET mice respectively, wile WT mice drank only 1.35 g/Kg. Measurements of ethanol preference show that both KO and ET mice display a statistically significant greater consumption of alcohol than water ($P<0.001$ and 0.05 respectively) compared to WT mice. Pre treatment of mice with GHB significantly reduced the amount of alcohol consumed in ET ($P<0.001$) mice but not in KO mice. The reduction in alcohol consumed was not due to the sedative effect of this low dose of GHB as confirmed by locomotor activity measurement.

These results show for the first time that the absence or reduced expression of GABAB receptors in KO and ET mice, respectively, significantly increases both the amount of ethanol consumed and the preference to ethanol, and suggests that GABAB receptors may play a pivotal role in controlling ethanol drinking behaviour. The action of GHB in reducing the amount of alcohol consumed appears to be mediated by GABAB receptors since was not evident in GABAB KO mice.

P 59/09

EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF METHOXETAMINE ON BEHAVIOUR, MOOD AND REWARD

Zanda Mary Tresa[1], Antinori Silvia[1], Ciaccio Francesco[1], Mutti Anna[2], Mancini Laura[2], Padovani Laura[2], Scherma Maria[1], Aroni Sonia[1], Muntoni Anna Lisa[3], Fratta Walter[1], Fadda Paola[1], Chiamulera Cristiano[2], Fattore Liana[3]

[1]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari ~ Cagliari ~ Italy, [2]Neuropsychopharmacology Laboratory, Division of Pharmacology, University of Verona ~ Verona ~ Italy, [3]CNR Institute of Neuroscience-Cagliari, National Research Council, Italy ~ Cagliari ~ Italy

Methoxetamine (MXE) is a novel psychoactive drug perceived as safe by users despite its severe adverse effect.

In this study we provide the first evaluation of its effects on behavior, mood and reward.

We initially tested the effect of an acute intraperitoneal (i.p.) administration of MXE (0.5-5 mg/kg) in male rats. Data showed that MXE transiently but significantly affects motor activity in a dose- and time-related manner, with low and high doses inducing hyper- and hypomotility, respectively, during the first 20-min after administration. As compared to controls, MXE dose-dependently also decreases the startle amplitude of rats in the pre-pulse inhibition test, which provides an operational measure of sensorimotor gating reflecting the ability of an animal to successfully integrate and inhibit sensory information. At the highest dose tested (5 mg/kg), MXE induces transient analgesic effects after 30/45-min from administration, as revealed by tail flick and hot plate tests. Any dose of MXE does not induce anxiety-like state in the elevated plus maze (EPM) whereas when tested in the marble burying test (MBT), rats treated with MXE 0.5 and 1 mg/kg buried a significantly higher number of marbles than controls, suggesting an anxious and/or obsessive-compulsive trait. Moreover, in the forced swim test (FST), MXE 5 mg/kg reduces immobility and climbing while significantly increasing swimming activity, suggesting an antidepressant effect. Moreover, when tested in a self-administration (SA) substitution protocol, the intravenous (i.v.) dose of MXE 0.25 mg/kg fully substituted for KET 0.5 mg/kg (i.v.), with significant differences from saline but not from KET. Conversely, MXE 0.125 mg/kg (i.v.) showed a partial substitution only, while MXE 0.5 mg/kg (i.v.) did not substitute for KET. In addition, MXE (0.5 and 0.25 mg/kg, i.v.) induced a significant and time-dependent enhancement of dopamine extracellular levels with respect to basal values in the nucleus accumbens shell. Consistently, MXE (0.031-0.5 mg/kg, i.v. cumulative doses) stimulated the activity of dopamine neurons in the ventral tegmental area.

Altogether, our results indicate that MXE differentially alters spontaneous motor activity, and increases swimming time (FST) at high doses, while inducing repetitive/perseverative (obsessive-compulsive) behaviors (MBT) at low doses but not spatial anxiety (EPM). Moreover at appropriate doses MXE is able to substitute for KET in the SA paradigm suggests that it possesses rewarding effects, a notion supported by both neurochemical and electrophysiological data.

P 60/09

BEHAVIOURAL PHENOTYPING OF CONDITIONAL CEREBELLAR SPECIFIC CHD7-DEFICIENT MICE

Caruso Angela[1], Riegman Kimberley[2], Michetti Caterina[1], Sabbioni Mara[1], Basson Albert[2], Fernandes Cathy[3], Scattoni Maria Luisa[1]

[1]Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Department of Craniofacial Development and Stem Cell Biology, King's College ~ London ~ United Kingdom, [3]Institute of Psychiatry, Psychology and Neuroscience, King's College ~ London ~ United Kingdom

ASD are a group of neurodevelopmental disorders with early onset, characterized by deficits in two core symptoms (social and communicative deficits and stereotyped behaviours). While the etiology of autism is still unknown, the strongest evidence appears to be genetic. Haploinsufficiency for the gene encoding chromatin remodelling factor CHD7 is the major cause of CHARGE syndrome, a condition associated with autism-like behaviours. CHD7 is expressed in the population of proliferative granule cell progenitors (GCps) that drives postnatal growth and foliation of the cerebellum. Loss of CHD7 from GCps reduced proliferation and enhanced neuronal differentiation, resulting in cerebellar hypoplasia.

As cerebellar defects are observed in autistic patients, we have generated a conditional mouse line (Chd7 deletion in GCps from E12.5) with hypoplasia of the lobules VI-VII to determine the behavioural consequences of cerebellar defects.

In order to evaluate the precise onset of vocal and motor alterations, we have analysed ultrasonic vocalizations and spontaneous motor behaviours during the first two postnatal weeks in the Chd7 mutant line. Furthermore, to robustly assess the acquisition of motor and sensory abilities and define a neurodevelopmental trajectory, a separate cohort of mice has been evaluated daily between birth and P20 for the assessment of developmental milestones. At adulthood, we have assessed the presence of communicative, social and motor deficits and repetitive behaviours.

Our data show motor and coordination difficulties in Chd7 mutant pups and adult mice, in line with motor alterations seen in ASD and CHARGE syndrome patients, while social and repetitive behaviours appear unaffected.

These results suggest that cerebellar hypoplasia in GCp-specific Chd7 conditional knockout mice is associated with development delay and motor deficits, on the other hand it is not sufficient to cause significant deficits in social behaviours.

P 61/09

INTERACTION BETWEEN EARLY PSYCHOSOCIAL STRESS AND MAO-A ON PATHOLOGICAL AGGRESSION

Pardu Alessandra[1], Frau Roberto[1], Pes Romina[1], Fanni Silvia[1], Saba Pierluigi[1], Godar Sean[2], Scheggi Simona[3], Demontis Maria Graziella[3], Bortolato Marco[2], Devoto Paola[1]

[1]Dept. of Biomedical Sciences ~ Cagliari ~ Italy, [2]Dept. of Pharmacology and Toxicology ~ Lawrence ~ United States, [3]Dept. of Molecular and Developmental Medicine ~ Siena ~ Italy

Gene-environment interactions have been shown to play a critical role in the development of aggression and other neuropsychiatric disorders. Several independent studies have highlighted that pathological aggression in males is often linked to the interaction of early-life abuse and/or neglect with allelic variants associated with low activity of monoamine oxidase (MAO) A, the key enzyme for the degradation of brain serotonin (5HT) and norepinephrine (NE). MAO-A knockout (KO) mice have been shown to display overt aggressive reactions and perseverative behaviors.

The goal of our study was to explore the neural underpinnings of the interaction between early psychosocial stress and a newly-developed line of MAO-A hypomorphic (MAO-A(Neo)) mice with very low brain MAO A activity. These mice show lower levels of overt aggression than MAO A KO mice and are an excellent model for MAO-A deficient individuals.

To simulate a human condition of abuse during childhood, we reproduced in MAO-A(Neo) neonates and in WT control littermates an adverse environment by subjecting pups to maternal separation (MS) throughout the first 8 post-natal days (PND) of their life and injecting them with saline solution as additional stressor. MS pups were euthanized at PND 9 and cerebral areas (PFC, CV and BS) harvested to analyze their content in 5-HT, NE and dopamine (DA) or processed to evaluate 5-HT2A receptor expression with Western blotting techniques. A set of behavioral experiment were performed to asses the aggression proclivity of stressed MAO-A(Neo) in comparison with non-stressed counterparts and WT mice.

While MS did not significantly affect the aggressive behavior in either MAO-A KO or WT mice, the same manipulation resulted in a robust enhancement of fighting responses in MAO-A(Neo) mice making them aggressive as MAO-A KO counterparts. Furthermore, the behavioral alterations of stressed MAO-A(Neo) mice were accompanied by significant changes in prefrontal cortex expression of 5-HT2A receptors, but not in tissue levels of 5-HT, NE and DA.

These data fit epidemiological findings on the interaction of low-MAO A allelic variants and early stress in males with respect to the development of antisocial behavior; furthermore, our findings provide a powerful translational platform to investigate the pathophysiology of aggression on a highly isomorphic murine model. Further studies are ongoing to characterize the neurobiological bases of gene-environment-sex interactions on the development of aggression and other emotional disturbances in MAO-A(Neo) mice.

P 62/09

INVOLVEMENT OF GLUTAMATE NMDA RECEPTORS IN THE ACUTE, LONG-TERM, AND CONDITIONED EFFECTS OF AMPHETAMINE ON RAT 50-KHZ ULTRASONIC VOCALIZATIONS

Costa Giulia, Morelli Micaela, Simola Nicola

Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy

Rats emit 50-kHz ultrasonic vocalizations (USVs) in response to either pleasurable natural or pharmacological stimuli, and these USVs have emerged as a new behavioral measure for investigating the motivational properties of drugs. Earlier studies have indicated that activation of the dopaminergic system is critically involved in 50-kHz USVs emission. However, evidence also exists that non-dopaminergic neurotransmitters participate in this behavioral response.

To ascertain whether glutamate transmission plays a role in 50-kHz USVs emission stimulated by amphetamine.

Rats received five amphetamine (1–2 mg/kg, i.p.) administrations on alternate days in a test-cage, alone or combined with the glutamate N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (0.1–0.5 mg/kg, i.p.). Seven days after treatment discontinuation, rats were re-exposed to the test-cage to assess drug-conditioning, and afterwards received a drug challenge. USVs and locomotor activity were evaluated, along with immunofluorescence for Zif-268 in various brain regions and spontaneous alternation in a Y maze.

Amphetamine-treated rats displayed higher 50-kHz USVs emission and locomotor activity than vehicle-treated rats, and emitted conditioned vocalizations on test-cage re-exposure. Rats coadministered amphetamine and MK-801 displayed lower and dose-dependent 50-kHz USVs emission, but not lower locomotor activity, during repeated treatment and challenge and scarce conditioned vocalization compared with amphetamine-treated rats. These effects were associated with lower levels of Zif-268 after amphetamine challenge and spontaneous alternation deficits.

These results indicate that glutamate transmission participates in the acute, long-term, and conditioned effects of amphetamine on 50-kHz USVs, possibly by influencing amphetamine-induced long-term neuronal changes and/or amphetamine-associated memories.

P 63/09

ROMAN HIGH- AND LOW-AVOIDANCE RATS DIFFER IN THE EXPRESSION OF PERK IN THE CEREBRAL CORTEX AND AMYGDALA DURING AVOIDANCE LEARNING

Piludu Maria Antonietta[1], Rosas Michela[1], Sabariego Marta[2], Giugliano Valentina[1], Giorgi Osvaldo[1], Acquas Elio[1], Corda Maria Giuseppa[1]

[1]Department of Life & Environmental Sciences, University of Cagliari, Italy ~ Cagliari ~ Italy, [2]Department of Psychology, University of Jaèn, Jaèn, Spain ~ Jaèn ~ Spain

Roman high- (RHA) and low- (RLA) avoidance rats are psychogenetically selected for, respectively, rapid versus poor acquisition of two-way active avoidance behavior in a shuttle box. Their divergent performance is due to the different emotionality and coping strategy displayed in the face of aversive conditions, rather than to different cognitive functions. The two-way active avoidance test is a typical example of instrumental conditioning that can be used to study associative learning. Several brain areas are involved in fear/instrumental conditioning, including the auditory (A1) and visual (V1) cortices, which analyze and elaborate the sensorial perceptions, and the amygdala (AMYG), which is essential for the association of the conditioned stimulus (CS) and the unconditioned stimulus (US) in classical aversive conditioning, and controls the expression of fear responses. The brain areas involved in avoidance learning, develop neuronal plasticity, in which the pERK cascade plays a pivotal role.

Given the marked differences in the performance of RHA and RLA rats in the two-way active avoidance test, and considering the key role played by the pERK cascade in instrumental/fear learning, the present study was designed to compare pERK activation in the A1, V1 and AMYG of RHA versus RLA rats during the acquisition of avoidance behavior in the two-way active avoidance test.

RHA and RLA rats were assigned to one of three groups and each group was submitted to a 40 min session of the test: "BOX" (exposed only to the shuttle box); "CS" (exposed to a series of CS), and "SHOCK" (submitted to the full active avoidance task) and the number of pERK positive cells/area was counted in the brain areas of interest.

In the SHOCK group of the RHA line, but not in their RLA counterparts, a significant increment of pERK immunoreactivity vs the respective BOX group was observed in both, the A1 and V1. In contrast, we did not observe pERK activation upon avoidance learning in the central (CeA) and basolateral amygdale (BLA) of RHA rats.

These results suggest that avoidance learning is associated with an activation of the ERK cascade in both, the auditory and the visual cortices, whereas the CeA and BLA might be more involved in the consolidation rather than in the acquisition of avoidance learning. Finally, this study support the view that the Roman lines represent a useful model to study the different patterns of pERK expression induced by fear/instrumental conditiong.

P 64/09

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

Sanna Fabrizio, Piludu Maria Antonietta, Corda Maria Giuseppa, Melis Maria Rosaria, Giorgi Osvaldo, Argiolas Antonio

University of Cagliari ~ Cagliari ~ Italy

Roman High (RHA) and Low Avoidance (RLA) rats display opposite behavioral traits: RHA rats are active copers, impulsive and prone to abuse drugs while RLA rats are reactive copers, hyperemotional and prone to develop depressive-like symptoms. These differences are linked to differences in brain monoamine (mainly dopamine) function and neuroendocrine responses to stress. RHA and RLA rats differ also in sexual behavior, with RHA rats displaying higher motivation and better copulatory performance than RLA rats. Moreover, in the two lines sexual behaviour is differentially influenced by dopamine agonists and antagonists, suggesting a different sensitivity/density of dopamine receptors in brain areas involved in sexual behaviour.

Taken together and in keeping with the well known role of dopamine in the modulation of sexual behaviour, these findings raise the possibility that the differences observed in sexual behaviour between RHA and RLA rats can be due to differences in brain dopamine transmission.

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

The results show that the higher sexual motivation and better performance of RHA vs RLA rats occurred concomitantly with a higher dopamine release, as shown by the higher dopamine concentrations found in the NAs dialysate of RHA vs RLA rats. These differences between the two lines were greater in naive animals and persisted, although attenuated, in experienced animals.

These findings confirm that a different mesolimbic dopaminergic tone exists in RHA and RLA rats, which may be responsible, at least in part, for their different copulatory patterns. Moreover, as sexual behaviour is one of the most important sources of natural reward due its importance in the preservation of species, these results, taken together with those that show differences between the two lines in the mesolimbic dopaminergic function after the intake of natural or drug rewards, strongly support the validity to use these two rats lines as a model for the study of the genetic, neurochemical and behavioural factors at the basis of individual differences in motivated behaviour and its alterations and their interactions with different classes of stimuli as well.

P 65/09

SPATIAL LEARNING IN INDIVIDUALS UNDERGOING ALCOHOL DETOXIFICATION

Carito Valentina[1], Coriale Giovanna[2], Fiore Marco[1], Ceccanti Mauro[2]

[1]Institute of Cell Biology and Neurobiology, National Research Council (IBCN-CNR)/IRCCS S. Lucia Foundation ~ Rome ~ Italy, [2]Center for Alcohol Abuse (Centro Riferimento Alcologico Regione Lazio-CRARL), Department of Clinical Medicine, Sapienza University of Rome ~ Rome ~ Italy

Alcohol dependence is a major public health problem worldwide. Brain and behavioral disruptions are common features of alcohol addiction. Alcoholism has a higher prevalence among men, though in recent decades, the proportion of alcoholics' women has increased but with quite different responding to ethanol addiction compared to men.

The aim of the present study was to investigate the effects of alcohol detoxification in alcoholic's men and women on memory function, particularly on spatial learning.

Changes in spatial memory were investigated on 29 alcoholics' men and 10 women undergoing alcohol detoxification by using a virtual Morris maze task. As age-matched controls we recruited 29 men and 8 women among occasional drinkers without history of alcohol dependence and/or alcohol related diseases and with negative blood alcohol level at the time of testing.

We found that the responses to the virtual Morris maze are mostly impaired in men rather than in women. Notably men undergoing alcohol detoxification showed increased latencies in the first movement during the trials, increased latencies in retrieving the hidden platform and increased latencies in reaching the visible platform. These findings were associated with reduced swimming time in the target quadrant of the pool where the platform had been during the 4 hidden platform trials of the learning phase compared to controls.

Such increasing latency responses reveal motor control, attentional and motivational deficits due to alcohol detoxification are possible other alternative explanations for the impaired place learning in the probe trial.

P 66/09

MATERNAL IMMUNE ACTIVATION IN MICE: SHORT AND LONG TERM NEUROBEHAVIOURAL EFFECTS IN OFFSPRING

Vigli Daniele, Scattoni Maria Luisa, Palombelli Gianmauro, Canese Rossella, Ricceri Laura

Istituto Superiore di Sanità ~ Rome ~ Italy

Potential environmental risk factors for several neuropsychiatric disorders (e.g. schizophrenia and autism) include prenatal viral/bacterial infections. Thus rodent models of maternal immune activation (MIA) have been developed and widely used also in preclinical studies to test treatment effectiveness.

The present study investigated short and long-term neurobehavioural effects of mimicking a maternal viral infection with polyinosinic-polycytidylic acid (Poly I:C), a synthetic analog of double-stranded RNA.

Poly(I:C) was injected into pregnant C57BL/6J dams on gestational day 12.5. Neonatal behavioural responses (ultrasonic vocalizations and spontaneous motor patterns) were measured in pups (both sexes) on postnatal days 4, 7, 10; at adulthood, open field test, stereotypic marble burying behaviour, sociability in the three-chamber test, fear conditioning and extinction and prepulse inhibition (PPI) were assessed in both sexes. In vivo magnetic resonance imaging and spectroscopy (MRI and 1H-MRS, 4.7 T) were also used to evaluate brain morphology and function in littermates of the behaviourally tested animals. Data were analysed always considering the litter-effect.

Neonatal vocalizations were altered in Poly I:C pups: a reduction in vocalization rate was evident on pnd 10, with males more affected than females. Regarding to spontaneous motor responses, Poly I:C male pups show an hyperlocomotor profile, with higher levels of pivoting throughout the ages considered. Increased stereotyped rearing and jumping responses were evident during both open field and marble burying tests. Lack of the expected habituation profile in adult Poly I:C exposed mice and a less anxious profile were evident in the open field test. In the three-chamber test, Poly I:C treated female mice showed an increased sniffing response of the cage containing the novel stimulus. In the fear conditioning test extinction impairments were evident: two days after testing Poly I:C males showed a delay in the initial part of the session, Poly I:C females showed a extinction delay throughout the test only one week later. PPI results showed sensorimotor gating deficits in Poly I:C exposed mice.

As for MRI, Poly I:C female brain volume is decreased by 4% when compared to control females.
Prenatal immune activation induces behavioural alterations primarily in explorative/stereotyped motor domains with some deficits in behavioural flexibility evidenced in a fear extinction and PPI tasks, sparing other cognitive and social competences. Mainly this experimental model appears a useful tool to evaluate MIA as a risk factor for brain development, rather than animal model of a single neuropsychiatric disorder.

P 67/09

ADOLESCENCE VERSUS ADULTHOOD: DIFFERENTIAL EFFECTS OF DRUGS OF ABUSE ON MESOLIMBIC DOPAMINE TRANSMISSION

Corongiu Silvia[1], Dessì Christian[2], Cadoni Cristina[2]

[1]Department of Biomedical Sciences, Neuropsychopharmacology Section, University of Cagliari ~ Cagliari ~ Italy, [2]Institute of Neuroscience, National Research Council of Italy ~ Cagliari ~ Italy

Increasing evidences suggest a heightened vulnerability to drugs of abuse in adolescence. During this developmental time frame the brain undergoes extensive remodelling affecting particularly reward system. Such changes involve both mesocortical and mesolimbic pathways. There is evidence for a predominance of ventral striatum (approach system) relative to prefrontal cortex (regulatory system) that produce typical adolescent behaviors (risk-taking, novelty seeking etc.) but, although most of the studies suggest a delayed maturation of the PFC, it is still debated if dopaminergic transmission in the nucleus accumbens (NAc) of adolescents is hyper- or hypo-reactive. In rodent models, in spite of overwhelming studies on reward function, tested through conditioned place preference or self-administration paradigms, direct evidences on adolescent dopamine (DA) transmission responsiveness to drugs of abuse are limited.

The aim of our study was to evaluate if there are differences in mesolimbic DA transmission between adults and adolescents after administration of different drugs of abuse through in vivo microdialysis.

Male Sprague-Dawley rats of 5, 6, 7 or 10,11,12 weeks of age were implanted with dual probe, aimed at the shell and core of NAc and challenged with nicotine (0.4 mg/kg s.c.), Δ9-tetrahydrocannabinol (THC, 1.0 mg/kg i.p.), cocaine (10 mg/kg i.p.) or morphine (1.0 mg/kg s.c.) and extracellular DA levels monitored simultaneously with behaviour.

Although no significant differences were observed between adolescents and adults in basal DA levels, neither in the shell and core of NAc, adolescents showed different effects depending on the drug and on the age of exposure. While no differences were observed in DA transmission responsiveness, both in the shell and in the core of NAc, after morphine or nicotine administration, rats at 6 weeks of age showed greater increase of DA levels in the NAc shell following 1.0 mg/kg i.p. of THC compared to adult rats. Moreover 5 weeks animals appear to be less sensitive to the DA increasing effects of cocaine (10 mg/kg i.p.) compared to adolescents of 6 and 7 weeks and to adults.

While differences observed following THC and cocaine challenge might be explained respectively by changes occurring in endocannabinoid system during development and in DA uptake transporter (DAT) levels as reported by previous studies, these results add new insights in the development of the reward system during different stages of adolescence.

P 68/09

CHANGES IN GLUTAMATE RELEASE AND RELATED MOLECULAR MECHANISMS INDUCED BY CHRONIC MILD STRESS IN VULNERABLE AND RESILIENT RATS. MODULATION BY KETAMINE

Tornese Paolo, Musazzi Laura, Sala Nathalie, Brizzi Francesco, Racagni Giorgio, Popoli Maurizio

Laboratory of Neuropsychopharmacology and Functional Neurogenomics - Dipartimento di Scienze Farmacologiche e Biomolecolari, Center of Excellence on Neurodegenerative Diseases (CEND), Università degli Studi di Milano ~ Milano ~ Italy

Increasing evidence has associated dysfunction of the glutamate system with the pathophysiology of stress-related neuropsychiatric disorders. Clinical studies on depressed patients have shown consistent volumetric and functional changes in brain areas where glutamate neurons predominate, and preclinical studies showed that stress deeply affects glutamatergic transmission in the same regions. While acute stress was shown to rapidly enhance glutamate release and transmission, an effect blocked by chronic antidepressants, the effect of chronic stress on glutamate release is still largely unknown.
Intriguingly, the NMDA-R antagonist ketamine (KET) was consistently reported to induce a rapid and sustained antidepressant effect. However, it is not clear if and how KET modulates glutamate release.

Using a Chronic Mild Stress (CMS) rat model of depression, we aimed to study the effects of chronic stress (and KET) on glutamate release and on synaptic and intracellular molecular mechanisms involved in the stress response.

Rats were exposed for 5 weeks to a variable sequence of mild unpredictable stressors, thus leading to a depressive-like state. To validate the CMS paradigm and separate susceptible from resilient rats, we performed the Sucrose Preference Test for anhedonia. Weight gain, adrenal/body weight and serum corticosterone levels were also measured to evaluate phenotypic changes induced by stress. 10 µg/kg KET was administered i.p. acutely 24 hours before sacrifice.
We measured basal and depolarization-dependent glutamate release from purified hippocampus (HPC) and prefrontal and frontal cortex (PFC/FC) synaptic terminals (synaptosomes) in superfusion.
Changes in the expression and phosphorylation levels of selected proteins in total homogenate, synaptosomes and synaptic membranes from HPC and PFC/FC were measured by Western Blot.

We found that chronic stress induces significant phenotypic changes in all rats subjected to the CMS protocol, and anhedonic behavior selectively in vulnerable rats. Selective changes in both basal and depolarization-evoked releases of glutamate were measured in HPC and in PFC/FC and acute KET partially reversed this changes. Finally, we found that both CMS and KET influence the expression and phosphorylation of different proteins involved in the regulation of presynaptic glutamate release and in the stress response (e.g.: metabotropic glutamate receptor 2, glucocorticoid receptor, mineralocorticoid receptor, synapsin I, cofilin).

Our results suggest that chronic exposure to stressful stimuli may induce alterations in glutamate release. Further investigation of the mechanisms regulating individual resilience or vulnerability to stress could help to clarify the physiopathology of depression and identify new pharmacological targets for faster and more efficient antidepressant therapy.

P 69/09

TIME-DEPENDENT FUNCTIONAL, STRUCTURAL, AND BEHAVIORAL CHANGES INDUCED BY ACUTE STRESS IN RATS. LOOKING FOR NEW TARGETS FOR NEUROPSYCHIATRIC THERAPIES

Sala Nathalie[1], Musazzi Laura[1], Tornese Paolo[1], Nava Nicoletta[2], Treccani Giulia[3], Bazzini Chiara[1], Sala Francesca[1], Luciano Roberta[1], Racagni Giorgio[1], Nyengaard Jens Randel[2], Wegener Gregers[3], Popoli Maurizio[1]

[1]Lab. of Neuropsycopharmacology and Functional Neurogenomics, Dipartimento di Scienze Farmacologiche e Biomolecolari and CEND, Università degli Studi di Milano, Milan, Italy ~ Milano ~ Italy, [2]Stereology and Electron Microscopy Laboratory, Centre for Stochastic Geometry and Advanced Bioimaging, Aarhus University Hospital, Aarhus C, Denmark ~ Aarhus ~ Denmark, [3]Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark ~ Aahrus ~ Denmark

Stress is a main risk factor in the etiopathogenesis of neuropsychiatric disorders. However, depending on the severity and duration of the stressor, stress may induce different and frequently opposite changes. Indeed, while acute stress initially often enhances neuronal activity and improves cognitive behavior, delayed effects of acute stress (as well as of chronic stress) induce morphological and functional impairments, particularly in brain areas where glutamate neurons are predominant. In this context, the study of time-dependent effects of stress on glutamatergic system could be crucial to understand how the initial pro-adaptive effects of stress might become maladaptive, increasing the risk of developing neuropsychiatric disorders.

Main aim of the study was to evaluate the time-dependent alterations induced by acute stress at functional, morphological and behavioral levels.

Male rats were sacrificed at different time points after the beginning of a standard footshock stress paradigm. Depolarization-evoked and hypertonic sucrose-evoked (measuring the size of the ready releasable pool, RRP) glutamate releases were assessed using the technique of purified prefrontal and frontal cortex (PFC/FC) synaptosomes in superfusion. The number of synapses within PFC was studied by means of serial section electron microscopy, while assessment of dendritic arborization and spine number was performed after Golgi staining. Cellular effectors were measured using western blotting on PFC/FC subcellular fractions. Working memory was assessed subjecting control and stressed rats to the delayed alternation T-maze test.

We found that acute stress induced early and sustained increase of glutamate release and RRP in PFC/FC up to 6 hours later. At morphological level, the number of PFC excitatory (selectively non-perforated and axospinous) synapses was significantly and markedly increased immediately after the stress session, and the number of synaptic spines was increased 24 h after stress, while apical dendrites arborization was decreased 2 weeks later.
Moreover, we measured time-dependent changes in the expression and phosphorylation levels of some molecular effectors regulating glutamate release and transmission and involved in the stress response (i.e. glucocorticoid receptor, mGluR2, synapsin I). Finally, behavioral tests showed that

acute stress improves working memory 2 hours later, but exerts an opposite effect 24 hours after stress.

The different glutamatergic modifications in PFC/FC functional and morphological plasticity suggest a biphasic process, during which the stress response may turn from early increased excitatory activation into its opposite. Deeper investigation of the effectors of this switch may help the discovery of new therapeutic targets for stress-related disorders.

P 70/09

EFFECTS OF 25I-NBOME, A NEW POTENT 5HT2A AGONIST, ON THE CENTRAL NERVOUS SYSTEM OF RODENTS

Miliano Cristina[1], Ossato Andrea[2], Marti Matteo[2], Loi Barbara[3], Mulas Giovanna[1], Carta Anna Rosa[1], Di Chiara Gaetano[1], De Luca Maria Antonietta[1]

[1]Department of Biomedical Sciences, Neuropsychopharmacology Section, University of Cagliari ~ Cagliari ~ Italy, [2]Department of Life Sciences and biotechnology (SveB), University of Ferrara ~ Ferrara ~ Italy, [3]School of Life and Medical Sciences,University of Hertfordshire ~ Hertfordshire ~ United Kingdom

25I-NBOMe(4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine), is a new psychoactive synthetic compound. It is available as “legal” alternative to LSD in Italy, but in other countries such as UK, Sweden, Serbia, USA, Russia, and Australia, it was banned. It is usually ingested sublingually and users are often unaware of ingesting fake LSD, so several episodes of acute intoxication have been reported with severe effects such as confusion, hypertension, tachycardia, hyperthermia, heart failure, generalized seizure, loss of consciousness, acute kidney injury. 25I-NBOMe acts as a partial agonist of 5HT2A receptor (Ki=0,087 nM), a Gprotein-coupled receptor that mediates the primary effect of hallucinogenic drugs. 5HT2A receptors are highly expressed in the prefrontal cortex, but also localized in some regions like the NAc shell and the dorsal striatum (CPu) which are involved in motivational and motor functions, respectively.

The aim of this study was to evaluate the abuse potential of 25I-NBOMe and its possible behavioural and neurotoxic effects on the central nervous system of rodents.

By in vivo microdialysis studies in rats we evaluated the effect of 25I-NBOMe (0.3-1.0 mg/kg ip) on dopaminergic (DA) transmission by implanting vertical probes in three dopaminergic terminal areas: medial prefrontal cortex (mPFC), nucleus accumbens (NAc) shell and core.
TH- and DAT-immunoreactive fibers analysis were performed in mice in coronal sections from the dorsal striatum (CPu) and ventral striatum (NAc Shell), after a sub-acute administration of 25I-NBOMe (2 mg/kg i.p.) ,once a day for 3 consecutive days.
Behavioural tests were performed in mice to provide information about the effects of 25I-NBOMe (0.1-1 mg/kg ip) on locomotor activity (open field, drag test and rotarod) and temporal expectation (reaction time task).

Microdialysis studies suggest this compound does not affect the DA transmission in NAc shell and core, neither in mPFC at the doses tested.

Spontaneous locomotory activity, Drag test and Accelerod test results showed no significant differences between animals treated with vehicle and animals treated with 25I-NBOMe. In the reaction time task, instead, animals exhibited a significant increase of reaction time within 30 minutes after the administration of 25I-NBOMe for all the three doses tested.
Microscopy analysis results showed there were no significant modifications in TH and DAT-ir fibers density in CPu and NAc shell.

Taken together the present results suggest that 25I-NBOMe does not show relevant pharmaco-toxicological central effects on DA transmission. Further experiments following different doses and repeated administrations are in progress.

P 71/09

EFFECTS OF WITHANIA SOMNIFERA DUNAL ON ETHANOL- AND MORPHINE-ELICITED CONDITIONED PLACE PREFERENCE IN MICE

Rosas Michela[4], Porru Simona[4], Spina Liliana[4], Longoni Rosanna[4], Kasture Sanjay[2], Ruiu Stefania[1], Orrù Alessandro[1], Peana Alessandra[3], Acquas Elio[4]

[1]Institute of Translational Pharmacology, UOS of Cagliari, National Research Council, Scientific and Technological Park of Sardinia - Polaris, Pula, Italy ~ Pula ~ Italy, [2]Sanjivani College of Pharmaceutical Education & Research, Kopargaon, India ~ Kopargaon ~ India, [3]Dept. of Chemistry and Pharmacy, University of Sassari ~ Sassari ~ Italy, [4]Dept. of Life and Environmental Sciences, University of Cagliari ~ Cagliari ~ Italy

The conditioned place preference (CPP) method allows the characterization of the motivational properties of drugs and is usefully applied either to investigate such properties of novel compounds and to establish the receptor-mediated mechanisms responsible of place conditioning. Accordingly, addictive drugs, such as ethanol and morphine, have been shown able to elicit a conditioned preference for the associated environment. Notably, in the field of drug addiction, there is a growing interest toward the application of extracts of herbal origin to interfere with the motivational effects of addictive substances. Among the plants extracts that may have a potential usefulness in this respect, the standardized methanolic root extract of Withania somnifera Dunal (WSE) has recently been reported to be of potential interest as it has been shown to interact with the effects of ethanol (ethanol withdrawal-induced anxiety, ethanol-induced anxiolysis and ethanol self-administration) and morphine (tolerance and dependence in chronically treated mice, reduction of dendritic spines density in the rat nucleus accumbens shell upon morphine withdrawal and morphine-induced hyperalgesia).

Aims of our research were the characterization of the effects of WSE on ethanol (2 g/kg)- and morphine (10 mg/kg)-elicited place conditioning.

To this end, male CD-1 mice underwent ethanol and morphine conditioning under standard procedures (4 pairings/day with the drug and 4 pairings/day with the vehicle). The effects of WSE (50 and 100 mg/kg) were evaluated on acquisition and expression of ethanol- and morphine-elicited CPP by administering it either before conditioning (acquisition) and before testing (expression). Furthermore, in order to exclude the possibility that WSE affects the expression of drug-elicited CPP by an action on spatial memory, the effects of WSE (50 and 100 mg/kg) were also studied in the two-trial memory recognition task.

The results of these experiments reveal that the administration of WSE significantly impairs both the acquisition and the expression of ethanol- and morphine-elicited CPP without affecting spatial memory. These results provide further support to the suggestion that the use of WSE could represent an interesting phytotherapeutic approach worth of further investigations for the treatment of excessive alcohol drinking and to prevent alcohol relapse and also point to WSE as an interesting tool in opiate addiction.

P 72/09

EFFECTS OF WITHANIA SOMNIFERA DUNAL ON ETHANOL SELF-ADMINISTRATION IN RATS

Acquas Elio[2], Rosas Michela[2], Porru Simona[2], Spina Liliana[2], Kasture Sanjay[1], Peana Alessandra[3]

[1]Sanjivani College of Pharmaceutical Education & Research, Kopargaon, India ~ Kopargaon ~ India, [2]Dept. of Life and Environmental Sciences, University of Cagliari ~ Cagliari ~ Italy, [3]Dept. of Chemistry and Pharmacy, University of Sassari ~ Sassari ~ Italy

The uncontrolled use of alcoholic beverages represents a serious risk factor for a great number of diseases and premature deaths. However, in spite of intense research on the mechanisms by which ethanol affects behaviour and may cause the development of alcoholism, an enormous gap has yet to be filled in order to provide pharmacological tools that may significantly impair these mechanisms. In this regard, recent investigations have pointed to Pueraria lobata (Kudzu), Panax ginseng and Salvia miltiorrhiza, as herbal remedies of some interest that have been investigated to be suggested as possible adjuvants for prevention and treatment of alcoholism. In addition to these plants, also Withania somnifera Dunal, a herbal remedy used in traditional medicine for its anti-inflammatory properties, has been reported to affect some effects of ethanol such as ethanol withdrawal-induced anxiety (reduction) and ethanol-induced anxiolysis (potentiation).

To investigate the effects of Withania somnifera roots extract (WSE) on motivation for drinking ethanol by operant self-administration paradigms.

Male Wistar rats were trained to self-administer ethanol (10%) by nose-poking. The effects of WSE (25 and 75 mg/kg) were evaluated on acquisition and maintenance of ethanol self-administration, on ethanol breakpoint under a progressive-ratio schedule of reinforcement, on ethanol deprivation effect and on reinstatement of seeking behaviour.

The results demonstrate that the administration of WSE significantly reduces acquisition, maintenance and breakpoint of ethanol self-administration as well as the deprivation effect and reinstatement of ethanol-seeking behaviour.

Further studies are necessary to characterize the individual compounds present in the extract that may have been responsible of these effects and to investigate in detail the mechanism(s) by which WSE interferes with the motivational properties of ethanol and affects ethanol taking behaviour. Overall, these results provide support to the suggestion that the use of WSE could represent an interesting phytotherapeutic approach for the treatment of excessive alcohol drinking and to prevent alcohol relapse.

P 73/09

ROLE OF PHOSPHORYLATED ERK IN THE MOTIVATIONAL PROPERTIES OF ETHANOL AS DETERMINED BY CONDITIONED PLACE PREFERENCE AND CONDITIONED PLACE AVERSION

Spina Liliana[1], Rosas Michela[1], Porru Simona[1], Longoni Rosanna[1], Peana Alessandra[2], Acquas Elio[1]

[1]Dept. of Life and Environmental Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]Dept. of Chemistry and Pharmacy, University of Sassari ~ Sassari ~ Italy

Place conditioning allows to investigate associative learning and their underpinning neurobiological mechanisms. Conditioned place preference (CPP) is based on the establishment of positive memories and as such allows to model drug-seeking behaviour, whereas conditioned place aversion (CPA) is based on the establishment of negative memories and results in the prevention of aversive experiences previously conditioned to drug-associated stimuli. The Mitogen-activating Extracellular Kinase (MEK)/Extracellular signal Regulated Kinase (ERK) cascade has been involved in associative learning and in place conditioning studies. However, while the role of MEK has been shown to be critical for the acquisition of drug-elicited CPP, further investigations are needed to characterize its role in CPP expression as well as in both acquisition and expression of drug-elicited CPA.

This study was aimed at investigating whether MEK blockade, with the brain penetrant MEK inhibitor, SL327, could impair acquisition and expression of ethanol (2 g/kg)-elicited CPP and CPA in male CD-1 mice.

To this end mice underwent ethanol conditioning under two distinct schedules: in backward conditioning experiments ethanol was administered before mice were placed in the conditioning apparatus (CPP) while, in forward conditioning experiments, ethanol was administered immediately after removing mice from the apparatus (CPA). In the acquisition experiments, SL327 was administered 60 min before ethanol whereas, in the expression experiments, SL327 was administered 60 min before the post-conditioning test.

The results demonstrate that ethanol elicited both a significant CPP and CPA and that SL327 significantly prevented the acquisition of ethanol-elicited CPP but not CPA; in addition, SL327 while able to reduce the expression of ethanol-elicited CPP, failed to prevent the expression of ethanol-elicited CPA.

These results extend previous data on the differential role of MEK on acquisition and expression of place conditioning and suggest that their activation may be at the basis of different mechanisms as a function of the motivational sign of the unconditioned stimulus (preference vs aversion) and of the experimental phase (acquisition vs expression).

P 74/09

EFFECT OF L-DOPA ON COCAINE-TAKING AND COCAINE-SEEKING BEHAVIOR IN RATS

Antinori Silvia[1], Fattore Liana[2], Saba Pierluigi[1], Fratta Walter[1], Gessa Gian Luigi[1], Devoto Paola[1]

[1]Division of Neuroscience and Clinical Pharmacology, Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]CNR Neuroscience Institute-Cagliari – National Research Council ~ Cagliari ~ Italy

Clinically, direct and indirect dopamine agonists have been suggested as therapeutic tools for cocaine addiction. Positive results were obtained with L-DOPA, the dopamine metabolic precursor that acts as indirect dopamine agonist. However, there are not preclinical studies about L-DOPA effects on animal models of drug self-administration.
Recently, we demonstrated that L-DOPA systemic administration suppressed cocaine-induced reinstatement of cocaine-seeking behavior in rats, and that this effect was mediated by a supra-maximal stimulation of dopamine D1 receptors that led to their functional inactivation.

In this study, we verified whether L-DOPA suppressed cue-induced reinstatement of cocaine-seeking behavior as effectively as cocaine-induced reinstatement.

Male Sprague Dawley rats were trained to self-administer cocaine (0.5 mg/kg/infusion) intravenously for 2 daily hours under a continuous (fixed-ratio 1, FR1) schedule of reinforcement, in which every active lever pressure corresponds to one cocaine infusion each associated with a visual and an auditory cue. Stimuli associated with contingent drug presentation play a key role in the reinstatement of drug-seeking in animals as in humans. After reaching a stable drug intake and following a prolonged extinction training, animals underwent cue-induced reinstatement testing, and received an intraperitoneal (i.p.) injection of L-DOPA (50 mg/kg) or its vehicle (saline) 20 minutes before starting the session.
Since extended access to cocaine self-administration may produce symptoms characteristic of addiction that are not seen following more limited access to the drug, after the first reinstatement test, the same animals were allowed to reacquire responding for cocaine during extended (6 hours) daily sessions; on the last day, rats were pretreated with L-DOPA (50 mg/kg, i.p.) to verify whether it was able to also affect cocaine-taking. Finally, after an extinction training, L-DOPA was tested on cue-induced reinstatement of drug-seeking behavior.

In the reinstatement test, a cue priming given immediately before starting the session promptly reinstated active lever pressing to a pre-extinction responding level, an effect significantly reduced by administration of L-DOPA at the same dose (50 mg/kg) previously shown to be effective in preventing cocaine-induced reinstatement.
As expected, cocaine self-administration was readily reacquired. Moreover, L-DOPA did not affect cocaine intake and was effective in suppressing cue-induced reinstatement of cocaine-seeking behavior.

Overall, our findings show that L-DOPA does not affect cocaine intake in rats, but since it is able to prevent both drug- and cue-induced reinstatement of cocaine-seeking behavior it might be useful in the suppression of relapse to cocaine seeking.

P 75/09

EFFECT OF 5-ALPHA REDUCTASE INHIBITION IN L-DOPA-INDUCED DYSKINESIA

Fanni Silvia[1], Frau Roberto[1], Tronci Elisabetta[2], Fidalgo Camino[2], Lisci Carlo[2], Pardu Alessandra[1], Pes Romina[1], Saba Pierluigi[1], Devoto Paola[1], Bortolato Marco[3], Carta Manolo[2]

[1]Dept. of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology ~ Cagliari ~ Italy, [2]Dept. of Biomedical Sciences, Section of Physiology ~ Cagliari ~ Italy, [3]Dept. of Pharmacology and Toxicology, School of Pharmacy ~ Lawrence ~ United States

Evidences show that neurosteroids regulate dopamine (DA) neurotransmission. Accordingly, we previously reported that inhibition of 5α-reductase (5AR), the rate-limiting enzyme for neurosteroids synthesis, elicits anti-DAergic actions. In particular, we found that in rodents, the 5AR inhibitor finasteride (FIN) is able to restore PPI deficits, hyperactivity and stereotyped responses induced by dopaminomimetic agents. Interestingly, all these behavioral effects were mediated by post-synaptic DAergic regulations in the striatum and were not associated to extrapyramidal symptoms.
Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra and consequential loss of dopamine functions in the striatum. L-DOPA the drug of election in PD, has been associated with dyskinesia onset, the most debilitating motor complication associated with chronic dopamine replacement therapy in PD.

As L-DOPA-induced dyskinesia (LID) is closely related to dysregulation of DA signaling in the striatum, the aim of this study was to investigate whether DA receptor modulation by neurosteroids would impact on the development and the expression of LID. Thus, we investigated the effects of 5AR blockade by FIN on LID in 6-OHDA-lesioned rats as model of PD.

Male and female lesioned rats were subjected to chronic L-DOPA administration until stable expressions of dyskinesia was achieved. Animals were divided in homogeneous groups based on Abnormal Involuntary Movement score (AIMs). Then, rats were acutely and chronically treated with different doses of FIN or its vehicle. In addition, in order to further evaluate the impact of FIN on DA receptor supersensitivity in the striatum, the effect of FIN was also assessed on dyskinesia induced by the D1/D2 receptors agonist apomorphine.

Results indicated that acute injection of FIN dose-dependently reduced the AIMs in both male and female 6-OHDA-lesioned rats. Despite the key therapeutic effects of FIN in humans rely on the conversion of testosterone in DHT, female rats appear to be more susceptible to FIN actions on LID. As observed after acute treatment, also chronic FIN injection elicited anti-dyskinetic effects, which were more pronounced in the first week of treatment. These results suggest that prolonged FIN exposure could give rise to partial drug tolerance. Finally, FIN was also able to dampen the apomorphine-mediated dyskinesia in both males and females.

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

P 76/09

NEUROCHEMICAL MECHANISMS INVOLVED IN MDMA-INDUCED NEUROTOXICITY IN MICE

Costa Giulia, Simola Nicola, Morelli Micaela

Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari ~ Cagliari ~ Italy

Several preclinical reports indicate that the amphetamine-related drug 3,4-methylenedioxymethamphetamine (MDMA) behaves as a dopaminergic neurotoxin in mice.

To clarify the mechanisms of such toxicity, this study evaluated the effects of MDMA on two markers of dopaminergic function, the dopamine transporter (DAT) and the enzyme tyrosine hydroxylase (TH), in the brain of mice during and after chronic-intermittent administration. Moreover, this study investigated the involvement of the serotonergic and GABArgic systems in MDMA-induced toxicity by evaluating the changes in glutamic acid decarboxylase-67 (GAD-67), neuronal nitric oxide synthase (nNOS), and serotonin transporter (SERT) in the mouse brain.

Different groups of mice received MDMA (10 mg/kg i.p.), twice a day/twice a week, from post-natal day (PND) 60 to PND 82 or 124, and were sacrificed at different time-points (PND 85-214) after the last administration, to obtain a time-course of effect.

The results obtained showed a reduction of DAT- and GAD-67-positive fibers in the striatum and medial prefrontal cortex (mPFC), and an increase in nNOS-positive neurons in the striatum, as early as 3 days after MDMA discontinuation. A similar time-course of effect was detected for the decrease in GAD-67-positive hippocampal neurons and TH-positive nigral neurons. Conversely, a reduction of striatal TH-positive fibers was observed only in mice treated from PND 60 to PND 124 and sacrificed on PND 138. Finally, SERT was never affected by the MDMA regimen used.

These results suggest that MDMA-induced dopaminergic neurotoxicity in mice is associated with GABAergic degeneration and increased nNOS expression, while serotonergic mechanisms appear not involved in this effect.

P 77/09

CHRONIC HYPOXIA UP-REGULATES CAV3.2 T-TYPE CALCIUM CHANNELS IN RAT SENSORY NEURONS

Calorio Chiara[1], Marcantoni Andrea[1], Comunanza Valentina[2], Bussolino Federico[2], Carbone Emilio[1]

[1]Department of Drug Science, University of Turin, Italy ~ Turin ~ Italy, [2]Laboratory of Vascular Oncology, Institute for Cancer Research and Treatment, 10060 Candiolo, Turin, Italy ~ Turin ~ Italy

T-type calcium channels (TTCCs) regulate pain sensation at peripheral (DRG neurons) and central levels. Their up-regulation is among the causes of hyperalgesia, as shown in streptozocin-induced diabetic rats, in which Cav3.2 channels are effectively up-regulated. These conditions are likely to be caused by the chronic local hypoxia at which peripheral nerves are steadily maintained. On the other hand, chronic hypoxia (CH) is known to up-regulate Cav3.2 channels both in adrenal chromaffin cells and PC12 cells through the activation of hypoxia-inducible transcription factor.

The principal aim of this work is to investigate whether chronic hypoxia (3% of O2 for 15-18h) has an up-regulatory action on Cav3.2 TTCCs and possibly alters their gating properties. A second primary aim is to identify the signaling pathway by which CH induces the up-regulation of TTCCs.

Electrophysiological measurements (current clamp and voltage clamp conditions) and immunostaining were performed on primary culture of rat DRG neurons.

We found that CH significantly increases the percentage of small DRG neurons expressing TTCCs (from 66 to 84%) without affecting their gating properties and the size of high-voltage activated Ca2+ currents. CH potentiates also DRG excitability, by increasing: a) the percentage of DRG neurons that exhibit an after-depolarization potential (ADP) during action potentials recordings (from 8% to 27%) and b) the firing frequency induced during different current injections without altering the neuronal firing adaptation.
Overnight incubations of the iron chelating agent deferoxamine (DFX), which activates HIFs, mimicked the up-regulatory effects of CH on TTCCs and immunostaining revealed an enhanced expression of HIF-1alpha in small-size DRG neurons exposed to CH. Patch-clamp experiments to identify the pathways of TTCCs up-regulation revealed the direct involvement of ROS species, PKC activation and IP3 -mediated calcium release from intracellular stores.

Our data reinforce the idea that TTCCs are pro-nociceptive and can be identified as potential therapeutic targets for the treatment of chronic pain associated with CH or any other cause of hyperalgesia.

P 78/09

HUMAN RECOMBINANT NGF EYE DROPS IN OPTIC NERVE CRUSH MODEL: MODULATORY AND REPARATIVE EFFECTS IN THE RETINA AND VISUAL BRAIN AREAS

De Nicolò Sara[1], Rosso Pamela[1], Mesentier--Louro Louise[2], Lambiase Alessandro[3], Rama Paolo[2], Tirassa Paola[1]

[1]National Research Council (CNR) Institute of Cell Biology and Neurobiology ~ Rome ~ Italy, [2]San Raffaele Scientific Institute, Division of Neuroscience, Eye Repair Unit ~ Milan ~ Italy, [3]Department of Sense Organs-Section of Ophthalmology, Sapienza University of Rome ~ Rome ~ Italy

Adult mammal Retinal ganglion cells (RGC) unable to spontaneously regenerate after axonal damage and reduced retrograde support of nerve growth factor (NGF) contribute to their loss. Recently, ocular application rhNGF as eye drops (E-rhNGF) has proved to exert protective and reparative action on ocular tissues in animals and humans and to extent its biological effects on brain areas receiving retinal projections, thus suggesting the application of E-rhNGF as a feasible means to counteract neurodegeneration throughout the visual system.

The present study was addressed to investigate the morphological and biochemical effects of E-rhNGF treatment in a rat model of unilateral optical nerve crush (ONC) on the retina and primary visual areas, such as the optic chiasm (OC), the lateral geniculate nucleus (LGN), the superior colliculus (SC) and the visual occipital cortex (VOC).

Long Evans male rats subjected to left ONC received 10µl of rhNGF (640µg/mL) or Saline as eye drops in the left eye immediately after crush and twice a day for the next 14 days post crush (14 dpc). Rats were killed at 7 and 14 dpc by intra-cardiac perfusion and by decapitation to dissect the ocular and brain tissues to be processed for immunostaining and western blot analysis using markers to evaluate RGCs and axon elongation, glutamate/GABA synaptic transmission, proNGF and NGF receptors.

E-rhNGF treatment to ONC rats results in increased RGC survival and axon growth, which were associated with a recovery of TrkA/p75NTR unbalance in both retina and retinal recipient brain areas. Changes of VGluT1 and VGlut2 and VGAT expression were also observed in LDG, SC and VCO of ONC and ONC+E-rhNGF rats indicating the involvement of synaptic transmission.

Confirming our previous studies, here it is demonstrated that E-rhNGF treatment following ONC activates regeneration and modulates retina and central visual pathways. A role of rhNGF in the regulation of regenerative program after injury and/or during ocular pathologic conditions is prospected.

P 79/09

VISUAL PROCESSING IN A MOUSE MODEL OF FOCAL NEOCORTICAL EPILEPSY

Vannini Eleonora[1], Panarese Alessandro[2], Mazzoni Alberto[2], Pietrasanta Marta[3], Lai Stefano[2], Micera Silvestro[2], Caleo Matteo[1], Restani Laura[1]

[1]CNR, Neuroscience Institute ~ Pisa ~ Italy, [2]Scuola Superiore Sant'Anna, Translational Neural Engineering Laboratory, The BioRobotics Institute ~ Pisa ~ Italy, [3]Scuola Normale Superiore ~ Pisa ~ Italy

Epilepsy is the second most common neurological disorder after stroke, affecting over 0.5% of the world population. A percentage of patients (30%) remain resistant to drug treatments. This pharmacoresistance predominantly affects patients with focal epilepsy, which are 40-50% of total incidence cases. Electrophysiological studies of refractory epilepsy are currently in progress to get insights into the mechanisms of circuit remodeling, thus paving the way for alternative therapeutic options.

We try to understand which are plastic rearrangements within the epileptic focus that trigger cortical dysfunction and hyperexcitability.

We employ a model of neocortical, non-lesional epilepsy based on local delivery of the clostridial enzyme tetanus neurotoxin (TeNT) in mouse visual cortex. TeNT is a metalloprotease that enters synaptic terminals and cleaves the synaptic vesicle protein VAMP/synaptobrevin, resulting in preferential blockade of inhibitory neurotransmission. We inject TeNT in primary visual cortex of adult mice and we recorded VEPs and single-units, also using multichannel linear probes (16 channels), which spans the whole cortical thickness, allowing a layer-specific analysis.

Delivery of TeNT to the adult cortex results in refractory epilepsy with electrographic seizures persisting for several months, even after the toxin has been cleared from the system (Mainardi, Pietrasanta et al. 2012). We recorded local field potentials and spiking activity both during baseline conditions and after presentation of visual stimuli, in control and TeNT-injected mice, at the completion of TeNT effects (i.e 45 days following injection). In TeNT-treated cortices we found that spontaneous neuronal discharge was increased and visual responses were less reliable, with a higher proportion of failure trials, associated to higher spiking activity before stimulus presentation. Electrophysiological and behavioral visual acuity (spatial resolution) was also consistently impaired in TeNT-injected mice. We also investigated how contrast-driven modulations in spiking activity and local field potential spectra were affected by TeNT injection. These analyses will shed light on circuit modifications associated to epileptic activity.

Our data contribute to elucidate neuronal mechanisms underlying the network hyperexcitability observed in neocortical focal epilepsy.

P 80/09

L-DOPA CHRONIC TREATMENT INCREASES REACTIVE MICROGLIA AND TNF- ALPHA PRODUCTION IN THE 6-OHDA LESIONED STRIATUM

Mulas Giovanna[1], Pillai Elisabetta[1], Espa Elena[1], Fenu Sandro[1], Lecca Daniela[1], Spiga Saturnino[2], Carta Anna Rosa[1]

[1]Dept. of Biomedical Sciences ~ Cagliari ~ Italy, [2]Dept. of Life and Environmental Sciences ~ Cagliari ~ Italy

L-DOPA is the most effective treatment for Parkinson's disease (PD). However, this therapy induces debilitating motor complications, including abnormal involuntary movements and dyskinesia. Neuroinflammatory processes are largely implicated in PD neuropathology and neuroinflammation may also be involved in the development of L-DOPA-induced dyskinesia (LID).

In the present study we investigate the role of neuroinflammation in the development of LID in the 6-OHDA rat model of PD.

Rats (280-320 g) were unilaterally injected into the left medial forebrain bundle with 6-hydroxydopamine. Two weeks after the lesion a group of hemiparkinsonian rats was chronically treated with pulsatile L-DOPA (DOPAp) (6 mg/kg/day s.c. for 15 days),while another group received chronic administration of continuous L-DOPA by osmotic minipumps (DOPAc) (12 mg/kg for 14 days) or vehicle. Moreover, one group of rats received an inflammatory insult with a single peripheral dose of Lipopolysaccharide (LPS, 2 mg/kg i.p.) 24 hrs before L-DOPA chronic treatment. Abnormal involuntary movements (AIMs) were evaluated on alternate days during the L-DOPA treatment as an index of dyskinetic responses, quantified as the time spent performing limb, axial, locomotor AIM. At conclusion of treatments, fluorescent immunoistochemistry for OX-42 immunoreactivity (IR), TNF-α-IR colocalization in OX-42(+) and NF- neurons, and iNOS-IR were performed in the dopamine-depleted dorsal striatum to assess the neuroinflammatory response, and analysed by confocal microscopy.

L-DOPAp-treated rats displayed a gradual development of AIMs, while DOPAc-treated rats did not develop any dyskinetic response. Moreover, rats preadministered with LPS before L-DOPA treatment showed a faster onset of limb and axial AIMs, and spent more time performing AIMs in each test, as compared to L-DOPA alone. 6-OHDA lesion induced an increase of all neuroinflammatory markers in the dorsal striatum. L-DOPAp-treated striatum displayed an increased OX-42-IR and iNOS-IR as compared to the lesioned vehicle-treated striatum, and microglia displayed an activated morphology. In addition, both microglia and neurons displayed increased levels of TNF-α colocalization. In contrast, L-DOPAc treated rats displayed OX-42-IR, iNOS and TNF-α levels comparable to the unlesioned vehicle-treated striatum. LPS pretreatment before chronic L-DOPA increased levels of OX-42-IR and iNOS-IR as compared to L-DOPA alone, and increased TNF-α colocalization in microglia, but not in neurons.

A neuroinflammatory response was associated with a dyskinetic L-DOPA regimen but not with a non-dyskinetic treatment. Moreover, a single peripheral inflammatory insult with LPS exacerbated both the dyskinetic responses and striatal inflammatory response, suggesting that neuroinflammation may contribute to the development of dyskinesia in PD.

P 81/09

TRANSACCADIC SPATIAL STABILITY AND PRE-SACCADIC PERCEPTION

Binda Paola[1], Cicchini Guido Marco[2], Burr David[3], Morrone Maria Concetta[4]

[1]Universita' di Pisa ~ Pisa ~ Italy, [2]Institute of Neuroscience CNR ~ Pisa ~ Italy, [3]Universita' di Firenze ~ Firenze ~ Italy, [4]IRRCS Stella Maris ~ Calambrone Pisa ~ Italy

Eye movements pose major problems to the visual system, because each new saccade changes the mapping of external objects on the retina. While continuously visible objects are perceived as stable in spite of the displacement of their retinal image, perisaccadic flashes are mislocalized – usually compressed toward the saccade target.

Our work aims at investigating the relationship between these two phenomena: trans-saccadic stability and peri-saccadic spatial distortions.

We ask normally sighted adult human participants to report the perceived position of briefly flashed visual probes. These are presented at about the onset of a saccade, either alone or accompanied by a “reference”: an identical briefly flashed stimulus appearing at a variable delay from the saccade. We perform measurements both in classic psychophysical set-up with stimuli presented on a CRT monitor, and in conditions that allow for precisely controlling the availability of spatial references: a dark anaechoic room, with a wide screen where visual stimuli can be presented against a featureless dark environment.

We show that a probe flashed just before saccade onset can be drawn toward another flashed stimulus (the reference) presented pre- -or post--saccadicaly. The interaction field is oriented along the retinotopic trajectory of saccade--induced motion, which suggests a spatiotopic mechanism for trans--saccadic integration. These behavioural measurements are consistent with the properties of “remapping cells” revealed by neurophysiological measurements on non-human primates. When the probe is presented alone, on the other hand, it is mislocalized in the direction of the saccade target, leading to the well known peri-saccadic compression of visual space. Both these phenomena are reliably observed in conditions where environmental cues provide plenty of spatial references, e.g. the borders of the display screen. In complete darkness, however, with acoustic--driven saccades and no visual references besides the localisation stimuli themselves, both the compression toward the saccade target and the interaction between perisaccadic flashes decreases dramatically.

Both flashed stimuli and stable visual references exert a powerful influence over perisaccadic localization, which can be interpreted as the signature of trans-saccadic spatial binding for the same object. This suggests a strong link between the transient illusions that affect our perisaccadic vision and the stability of perception for the rich visual scene we are usually exposed to. We speculate that this can be supported by mechanisms that can be related to the neural phenomenon of "spatial remapping".

P 82/09

VISUAL BOLD RESPONSE IN LATE-BLIND SUBJECTS WITH ARGUS II RETINAL PROSTHESIS

Castaldi Elisa[1], Cicchini Marco[2], Cinelli Laura[4], Biagi Laura[3], Tosetti Michela[3], Rizzo Stanislao[4], Morrone Concetta[1]

[1]Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa ~ Pisa ~ Italy, [2]CNR, Neuroscience Institute, Pisa, Italy ~ Pisa ~ Italy, [3]Department of Developmental Neuroscience, Stella Maris Scientific Institute ~ Pisa ~ Italy, [4]Azienda Ospedaliero-Universitaria Careggi, SOD Oculistica ~ Florence ~ Italy

The recent growing advance in retinal prosthesis technology is generating hope for partially restoring vision to blind people with retinal pathology. However, these strategies require that the visual system downstream of the retinal circuitry to be capable of transmitting and elaborating visual signals.

We aim to understand how visual cortical processing changes during the course of training of late blind subjects with Argus II Retinal Prosthesis, and whether the visual cortex retains the capability of plastic remodelling (necessary to adapt to restored visual inputs).

We assess the visual function of a group of 6 blind patients affected by Retinitis Pigmentosa before and after (1.5 year time interval) implantation. We measured motion direction discrimination (size 40° X 40°, spatial frequency 0.06 cpd, temporal frequency 1 Hz; Michelson contrast 60%), as well as detection of the same stimuli presented stationary to the operated and not operated eyes in 2AFC design. All subjects underwent prolonged perceptual learning training to use the incoming signal from the implant. We also recorded BOLD response before and after the implant in three subjects in response to full-field flashing stimuli (1Hz) alternated with rest periods of dark.

When the subjects used the prosthetic implant, they were all capable of achieving good detection, but only 3 out of 6 showed good direction discrimination. However, no subject showed any improvement of vision in either eye when not aided by the Argus II. The BOLD activity in the primary visual area and the lateral geniculate nucleus was very weak before the implant, but after surgery the response to incoming visual inputs was enhanced.

The increase of visual responses to flashing lights in LGN and V1 might indicate that these regions retain the capability to respond to visual input even after years of deprivation. This is the first study testing a functional recovery of visual areas in patients after retinal implant. These results suggest spared plasticity potential in adult brain and encourage further research to improve technology for vision restoration.

P 83/09

EXPERIENCE-DEPENDENT DNA METHYLATION REGULATES PLASTICITY IN THE DEVELOPING VISUAL CORTEX

Napoli Debora, Tognini Paola, Tola Jonida, Silingardi Davide, Floriana Della Ragione, D''Esposito Maurizio, Pizzorusso Tommaso

DNA methylation is an epigenetic modification that consists in the addition of a methyl (-CH3) group to the fifth carbon of cytosines. DNA methyltransferases (DNMT) catalyzes this reaction altering the transcription status of the genes. Although it has been considered static for long time, recently many works demonstrate that DNA methylation is dynamically regulated in postmitotic neurons by electrical activity,during learning and memory, circadian rhythm and drug addiction.

Our aim is to assess if ongoing sensory stimuli could modify the epigenetic status of neurons in mouse primary visual cortex to modulate the transcriptional program necessary for experience-dependent plasticity.

We employed ocular dominance plasticity (ODP) induced by monocular deprivation (MD) during critical period as model of brain plasticity. After three days of MD, mice were studied with electrophysiological recordings and molecular analyses. We used the methylated and hydroymethylated DNA Immunoprecipitation (MeDIP and hMeDIP) followed by Real time-PCR and bisulfite sequencing to assess the epigenetic status of two well-known plasticity genes. Next, DNMT activity was blocked by pharmacological inhibitor and gene expression changes were studied by real time and moreover, by RNA-sequencing.

MD increased DNMT expression and decreased the mRNA level of cofactors (GADD45a, GADD45b and GADD45g) involved in the demethylation pathway. In agreement with DNMT upregulation, we found an increase of DNA methylation on the promoter regions of two well-known plasticity genes: BDNF exon IV and mir132. Deprivation of light stimuli exerted opposite effects on hydroxylmethylation of these two promoters. According to the epigenetic status of their regulatory regions, we found a downregulation of BDNF and mir132 transcripts. Inhibition of DNMT activity by RG108 infusion blocked this downregulation and also the downregulation of other 45 genes, assessed by next generation sequencing. Finally, we electrophysiologically tested ODP in monocular deprived mice that were infused with RG108 and we found no changes in ocular dominance physiology.

Taken together these data suggest that visual stimuli can regulate the epigenetic status of DNA on specific regulatory regions modifying the transcriptional program necessary for the molecular processes underlying ODP. In conclusion, DNA methylation can be used as molecular mediator in the experience-dependent refinement of cortical circuits during development.

P 1/10

FUS PROTEIN IS MISLOCALIZED IN THE CYTOPLASM OF CULTURED SKIN FIBROBLASTS FROM PRECLINICAL FUS P525L MUTATION CARRIERS

Lo Bello Margherita[1], Di Fini Francesca[1], Conforti Francesca L[2], La Bella Vincenzo[1], Spataro Rossella[1]

[1]ALS Clinical Research Center, Dept of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, 90129 Palermo, Italy ~ Palermo ~ Italy, [2]Institute of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy ~ Cosenza ~ Italy

Symptom onset in Amyotrophic Lateral Sclerosis (ALS) typically occurs when already over 70% of motor neurons are lost. This suggests a relatively long preclinical phase, in which no cognitive, electrophysiological or neuroimaging changes are detectable (Eisen A et al, 2014). However, the molecular changes preceding ALS onset are largely unknown (Freischmidt A et al, 2014). The availability of genetic tests in patients and relatives now allows identification of preclinical carriers, which are of invaluable help for the understanding the molecular changes preceding the disease's clinical onset.

To study the expression of FUS protein in skin fibroblasts from asymptomatic, preclinical carriers of FUS P525L mutation, an healthy control and patients with sporadic ALS.

Skin fibroblasts from two preclinical sisters carrying a FUS P525L mutation, one healthy control and two patients with sporadic ALS, with no identified gene mutations, were cultured. The two carriers and the control were asymptomatic, while the two ALS patients fulfilled the El-Escorial criteria for definite ALS. Western blot and immunocytochemistry were performed with specific antibodies to study the expression and subcellular localization of FUS protein in the skin fibroblasts.

Western blot and immunocytochemical studies showed that, in sporadic ALS, FUS protein has an exclusive nuclear localization, where it forms aggregates. In the healthy control, FUS is mostly nuclear with a less intense cytoplasmic expression, whereas in the two P525L mutation carriers FUS in strongly expressed in both nucleus and cytoplasm. A significant proportion of fibroblasts from the two P525L carriers have an exclusive cytoplasmic FUS expression.

FUS protein is differentially localized in fibroblasts from FUS P525L mutation carriers, the healthy control and the patients with sporadic ALS. In the mutation carriers, FUS is partially mislocalized in the cytoplasm. This represents the first evidence of an important biochemical change occurring in cells taken from preclinical ALS FUS P525L mutation carriers, and suggesting a role of mislocalized FUS as molecular marker before disease's onset. The study of preclinical molecular changes in ALS offers a unique opportunity to design new drugs and /or preventive strategies aimed to rescue the damaged motor neurons.

P 2/10

CATESTATIN AND MUSCIMOL INTERACTION IN AMYGDALA AND BRAINSTEM OF SPONTANEOUSLY HYPERTENSIVE RATS EXERT ANTIHYPERTENSIVE AND NEUROPROTECTIVE EFFECTS

Avolio Ennio[1], Mahata Sushil K[2], Mele Maria[1], Alò Raffaella[1], Fazzari Gilda[1], Pasqua Teresa[3], Angelone Tommaso[3], Cerra Maria Carmela[3], Canonaco Marcello[1]

[1]Comparative Neuroanatomy Laboratory of Biology, Ecology and Earth Science Dept. (DiBEST) University of Calabria, Ponte P. Bucci 4B, 87030 Arcavacata di Rende, Cosenza, Italy ~ Arcavacata di Rende, Cosenza, ~ Italy, [2]VA San Diego Healthcare System/Department of Medicine University of California, San Diego, La Jolla, CA 92093-0838, USA ~ San Diego, La Jolla, CA 92093-0838, USA ~ United States, [3]Laboratory of Cardiovascular Pathophysiology, Ecology and Earth Science Dept. (DiBEST), University of Calabria 87036, Arcavacata di Rende, Cosenza, Italy. ~ Arcavacata di Rende, Cosenza ~ Italy

Hypertension is a major risk factor for cerebrovascular diseases leading to vascular dementia and neurodegeneration. The chromogranin A derived peptide catestatin (CST) exerts sympathoexcitatory and hypertensive effects when microinjected into the rostral ventrolateral medulla (RVLM: excitatory output) and sympathoinhibitory plus antihypertensive effects when microinjected into the caudal ventrolateral medulla (CVLM: inhibitory output) of the vagotomized normotensive rat.

It was our intention to establish the hypothesis that Cst (antihypertensive) acting at the brainstem and amygdalar level may interact with the GABAergic signal thereby determining a decrease in both blood pressure and neurodegeneration events accounting for vasodilation together with the improvement of cerebral blood flow. Moreover, test the hypothesis that the two molecules Cst and GABAergic agonists act on the same target (GABAA receptor), but also in a complementary manner, suggesting the existence of new targets for Cst and clarify if other mechanisms aside those mediated by GABAergic signaling are required for the effects of Cst and how these mechanisms may lead to hypertensive states.

To perform the experiments we used male SHRs aged 12-36 weeks (345.8±50.2 g). In particular we injected IC CST into the central amygdalar nucleus for 15 days and consequently we recordings with whole-cell patch-clamp on pyramidal CeA neurons postsynaptic currents amplitude plus frequency and rise time/decay time. Finally we used ACS to evaluate neurodegeneration and western blot to measure the eventual increase or decrease of anti-apoptotic factors as Akt and ERK.

Continuous infusion of CST into the central amygdalar nucleus for 15 days resulted in a marked decrease of blood pressure in 6 (by 37 mmHg) and 9 months (by 65 mmHg) old spontaneously hypertensive rats. Whole-cell patch-clamp recordings on pyramidal CeA neurons revealed that CST increased both spontaneous inhibitory postsynaptic currents amplitude plus frequency along with reductions of sIPSC rise time and decay time. Inhibition of GABAA receptors by bicuculline completely abolished CST-induced sIPSC corroborating that CST signals occur through this major neuroreceptor complex. We found marked neurodegeneration in the amygdala and brainstem of 9

months old SHRs, while CST and the GABAAR agonist Muscimol provided significant neuroprotection. Enhanced phosphorylation of Akt and ERK accounted for neuroprotective effects through anti-inflammatory and anti-apoptotic activities

Overall our results point to CST exerting potent antihypertensive and neuroprotective effects plausibly via a GABAergic output, which constitute a novel therapeutic measure to correct defects in blood flow control of disorders such as stroke and Alzheimer's disease.

P 3/10

DIFFERENTIAL MODULATION OF DOPAMINE-GLUTAMATE POSTSYNAPTIC INTERACTION BY CAFFEINE AND NICOTINE AND THEIR COMBINATION WITH ANTIPSYCHOTICS: RELEVANCE TO PSYCHIATRIC DISEASES

Buonaguro Elisabetta Filomena, Avvisati Livia, Latte Gianmarco, Fico Giovanna, Avagliano Camilla, Marmo Federica, Iasevoli Felice, Tomasetti Carmine, De Bartolomeis Andrea

Department of Neuroscience, Reproductive Sciences and Odontostomatology – University "Federico II" ~ Napoli ~ Italy

Caffeine and nicotine are legal psychoactive substances and their consumption/abuse is particularly common in patients with schizophrenia and bipolar disorder.

In this study we have evaluated the expression of genes and proteins of the postsynaptic density (PSD) in response to acute treatment with caffeine and nicotine, in comparison with haloperidol, a typical antipsychotic, and GBR12909, a cocaine –like psychotomimetic drug, as well as the combined treatment of caffeine and nicotine with haloperidol.

Male rats were assigned to the following experimental groups: Saline+Vehicle (SAL+VEH); Saline+Haloperidol 0.8mg/kg (SAL+HAL); Saline+GBR12909 30mg/kg (SAL+GBR); Saline+Caffeine 40mg/kg (SAL+CAF); Salina+Nicotine 1.5mg / kg (SAL+NIC); CAF+HAL; NIC+HAL. Drugs were dissolved in saline, adjusted to physiological pH and intraperiotoneally injected. The second injection was made 20min after the first one. After 150min, animals were sacrificed and brains were frozen at -80 °C. Half of the brains were dissected and lysed for Western Blotting (WB) analysis. The others were cut at the cryostat in order to obtain coronal sections of 12µ for in Situ Hybridization (ISHH).

Early genes such as Homer1a and Arc had a predominant expression in the dorsal striatum and nucleus accumbens after HAL administration and a typical expression pattern in dorsolateral and ventromedial striatum after GBR administration. CAF did not induce a striatal expression of the two genes and only modestly induced them in the cortex. NIC induced Homer1a and Arc in dorsomedial striatum. The combination of HAL with CAF and NIC completely disrupted the distribution of the two genes induced by the individual drugs: CAF+HAL induced Homer1a and Arc in the dorsal striatum, while NIC+HAL induced a wider pattern of expression in the ventral striatum compared to HAL. Homer1b/c was up-regulated in the CAF+NIC+HAL and HAL groups. WB analysis revealed an over-expression of Arc and Homer1a proteins in the CAF+NIC+HAL and HAL groups. There was also a modest down-regulation of D1receptors in the CAF+HAL group correlated with increased expression of mGluR5.

The data show that caffeine and nicotine are able to modulate genes involved in the dopamine-glutamate postsynaptic interaction. Their combination with antipsychotics induces a differential and synergistic modulation compared to that produced by individual drugs in specific brain areas. These results open the way for the definition of the molecular signature underlying the use of coffee and tobacco and their combined effects in psychiatric patients under treatment.

P 4/10

NICOTINIC ACETYLCHOLINE RECEPTORS CONTAINING ALFA5 SUBUNIT REGULATE DOPAMINE AND SEROTONIN BUT NOT ACETYLCHOLINE RELEASE IN NUCLEUS ACCUMBENS: AN IN VIVO MICRODIALYSIS STUDY USING ALFA5 KO MICE

Vilella Antonietta[1], Cannazza Giuseppe[2], Stasiak Natalia[1], Zoli Michele[1]

[1]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy ~ Modena ~ Italy, [2]Department of Life Sciences, University of Modena and Reggio Emilia , Via Campi 183, 41125 Modena, Italy ~ Modena ~ Italy

Identification of the biological mechanisms involved in the vulnerability to develop nicotine addiction represents a major challenge for nicotine addiction research and is the basis to allow novel efficient preventive and therapeutic strategies. These molecular processes are largely analyzed by using transgenic mice and genome wide association studies. Recently a strong association between a human haplotype encompassing the CHRNA3/4/B4 gene cluster and predisposition to high levels of smoking has been reported.
A particular single nucleotide polymorphism (SNP) was identified in the α5 gene (a5SNP) conferring a two-fold higher risk of developing tobacco addiction in people homozygous carriers of α5SNP compared to non-carriers.
In vitro and in vivo preclinical studies suggest that a5SNP is responsible of a partial loss of function of nAChRs containing α5 (α5*-nAChRs) resulting in a decreased sensitivity to nicotine-elicited reward.
Release of dopamine (DA) in the mesolimbic pathway is classically considered as a major molecular correlate of drug reinforcement.

In this study, we assessed whether mice lacking α5*-nAChRs, α5-/- mice have alterations in DA and other (serotonin, 5HT, acetylcholine, ACh) neurotransmissions in the nucleus accumbens (nAc).

These neurotransmissions were challenged with systemic nicotine and assessed by in vivo microdialysis.

No differences were found in mean baseline DA concentrations between WT (0.57±0.09 nM) and α5-/- mice (0.73±0.16 nM). Two-way repeated measures ANOVAs on DA outflow over time showed significant genotype effect and nicotine dose effect. Furthermore, DA perfusate levels during the first hour post-injection were significantly higher in response to nicotine at the doses of 0.2 mg/kg (p=0.025), 0.4 mg/kg (p=0.04) and 1 mg/kg (p=0.011) compared to saline in WT mice. In α5-/- mice, DA perfusate levels during the first hour post-injection were significantly higher in response to nicotine compared to saline only at the dose of 1 mg/kg (p=0.021). Similar results were observed for 5HT, while no significant change was observed in ACh perfusate levels.

Present data demonstrate a shift to the right in the systemic nicotine dose-response curve of DA release in nAc, and support the notion that α5 KO or loss-of-functionα5 polymorphisms decrease the sensitivity to nicotine thus predisposing to heavy smoking.

P 5/10

SIGNIFICANT CORRELATION BETWEEN LONG-TERM LITHIUM TREATMENT AND LEUKOCYTE TELOMERE LENGTH IN PATIENTS WITH BIPOLAR DISORDER

Pisanu Claudia[1], Baggiani Gioia[2], Congiu Donatella[1], Melis Carla[1], Niola Paola[1], Caria Paola[1], Severino Giovanni[1], Vanni Roberta[1], Bocchetta Alberto[2], Del Zompo Maria[1], Chillotti Caterina[2], Squassina Alessio[1]

[1]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]Unit of Clinical Pharmacology of the University Hospital of Cagliari ~ Cagliari ~ Italy

Bipolar disorder (BD) is a disabling psychiatric disease characterized by alternating episodes of mania and depression. Lithium represents the mainstay in the maintenance of BD, but its mechanism of action is still far from being completely elucidated. Several studies reported premature cell senescence in BD, as shown by reduced telomere length in affected subjects.
Recent findings have also shown that antidepressants and lithium may have a protective effect against telomere shortening.

In this study, we sought to investigate the correlation between leukocytes telomere length (LTL) and clinical response to long-term lithium treatment in BD.

The sample comprised 200 patients of Sardinian ancestry with BD diagnosed according to DSM-IV and SADS-L criteria. Number of manic and depressive episodes, duration of lithium treatment and number of suicide attempts were also assessed. Patients were characterized for lithium response using the “Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder” as described previously. DNA was extracted from leukocytes and relative LTL assessed using SYBR Green real-time PCR. Correlation between LTL and age was assessed using nonparametric Spearman's correlation test. Correlation between LTL and duration of lithium treatment was determined using the partial correlation test, controlled for age. The effect of duration of lithium treatment, number of suicide attempts, number of depressive and manic episodes on LTL was assessed using the linear regression test, controlled for age. Dependence of LTL on diagnosis or response to lithium treatment was tested using analysis of covariance (ANCOVA), adjusted for age.

LTL correlated negatively with age ($P = 0.0002$) and was independent of sex ($p > 0.05$). There was a trend for a longer LTL in BD I patients compared to BD II patients, although not statistically significant ($P = 0.062$). Interestingly, LTL correlated positively with lithium treatment duration in patients with a duration of lithium treatment above 24 months ($n = 150$, $p = 0.037$) and was positively dependent on lithium treatment duration (lithium treatment duration: $p = 0.037$; age: $p = 0.003$). LTL was not dependent on lithium response, number of suicide attempts and number of depressive or manic episodes, after adjusting for age.

Our data support previous findings showing that long-term lithium treatment has a protective effect against telomere shortening in BD patients, though in our study this effect appeared to be independent from lithium clinical efficacy.

P 6/10

DYSREGULATION OF GPR17, A NEW KEY ACTOR INVOLVED IN OLIGODENDROGENESIS, IN A RODENT MODEL OF MULTIPLE SCLEROSIS: IMPLICATIONS FOR RE-MYELINATION STRATEGIES

Coppolino Giusy Tindara[1], Lecca Davide[1], Marangon Davide[1], Viganò Francesca[2], Menichetti Gianluca[1], Fumagalli Marta[1], Bonfanti Elisabetta[1], Furlan Roberto[3], Dimou Leda[2], Abbracchio Maria Pia[1]

[1]Dept. of Pharmacological and Biomolecular Sciences, University of Milan ~ Milan ~ Italy, [2]Physiological genomics, Institute for Physiology and Ludwig-Maximilians University Munich ~ Munich ~ Germany, [3]San Raffaele Scientific Institute, Milan ~ Milan ~ Italy

GPR17 is a membrane receptor activated by uracil nucleotides and cysteinyl-leukotrienes, mediators involved in inflammatory responses in the CNS. Under physiological conditions, GPR17 is expressed in Oligodendrocyte Precursor Cells (OPCs), with maximal levels in immature oligodendrocytes and progressive downregulated in terminally differentiating cells. A marked GPR17 up-regulation has been found in rodent models of cerebral trauma, ischemia and in lysolecithin induced focal demyelination. Little is known about GPR17 alterations in a primary demyelinating disease such as multiple sclerosis (MS).

This work was aimed at characterizing GPR17 expression pattern in acute Experimental Autoimmune Encephalomyelitis (EAE) mice, a murine MS model.

EAE was induced in eight-week-old female C57BL/6 mice immunized with MOG35-55/CFA and treated with pertussin toxin (PTX) at 0 and 2 days post-immunization (DPI). EAE was also induced in an inducible fluorescent reporter GPR17-iCreERT2xCAG-GFP mouse line. Tamoxifen was administered 2 weeks before EAE induction. Disease severity was monitored daily. Animals were sacrificed at 21 DPI in order to perform immunohistochemistry (IHC) analysis and semi-quantitative real-time PCR (qRT-PCR) analysis.

As already described for brain, IHC analysis revealed that, also in spinal cord, GPR17 specifically decorates a subset of early OPCs both in grey and white matter. After EAE induction, although the total number of GPR17+ cells was reduced in spinal cord white matter (likely due to generalized tissue loss), numerous GPR17+ cells accumulated around demyelinating lesions in close vicinity to activated inflammatory cells (microglia and blood-derived infiltrated monocytes/macrophages). In particular, the number of GPR17/NG2-positive cells was increased in the white matter, suggesting a disease-induced recruitment and proliferation of early progenitors. Real-time PCR analysis confirmed GPR17 up-regulation in the same group of animals. The availability of an inducible reporter GPR17-iCreERT2xCAG-GFP mouse line for fate mapping studies recently allowed us to directly visualize the behaviour of GPR17+ cells in the acute EAE phase. Data confirmed a strong increase of GFP+ cells at the sites of demyelinating lesions.

Characterization of the molecular defects of GPR17 in EAE will help re-establishing its correct function in remyelination and foster the identification of new pharmacological strategies to enhance the reparative potential of OPCs in the adult spinal cord.

P 7/10

INHIBITION OF MONOACYLGLYCEROL LIPASE ACTIVITY MODULATES THE ACTIVATION OF BRAIN STRUCTURES RELEVANT FOR MIGRAINE PATHOGENESIS

Greco Rosaria[1], Bandiera Tiziano[2], Mangione Antonina Stefania[1], Demartini Chiara[1], Nappi Giuseppe[1], Sandrini Giorgio[3], Blandini Fabio[4], Piomelli Daniele[2], Tassorelli Cristina[3]

[1]Laboratory of Neurophysiology of Integrative Autonomic Systems, Headache Science Centre, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [2]Drug Discovery and Development department, Istituto Italiano di Tecnologia, Genova, Italy ~ Genova ~ Italy, [3]Laboratory of Neurophysiology of Integrative Autonomic Systems, Headache Science Centre, "C. Mondino" National Neurological Institute;Dept. of Brain and Behavioural Sciences, University of Pavia ~ Pavia ~ Italy, [4]Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, “C. Mondino” National Neurological Institute ~ Pavia ~ Italy

Experimental evidence shows that the anti-nociceptive action of endocannabinoids, related to the modulation of the trigeminovascular system activity, may be helpful for prompting new targets for the treatment of migraine. URB602 is an inhibitor of monoacylglycerol lipase (MAGL), a key enzyme in the hydrolysis of the endocannabinoid 2-arachidonoylglycerol (2-AG). URB602 induces analgesia in animal models of pain not related to migraine, but there is no pre-clinical information as regards its potential effect in migraine pain.

To evaluate whether URB602 administration interferes with the level of activation of brain structures involved in migraine.

Nitroglycerin (NTG) induces neuronal activation in a specific subset of brain nuclei that are considered relevant for the development of migraine attacks. In this study we evaluated the changes caused by URB602 in NTG-induced neuronal activation. Male Sprague Dawley rats were treated with NTG (10mg/kg, i.p.) followed by URB602 (2mg/kg, i.p.) or vehicle (DMSO, 1ml/kg, i.p.). Their brain were processed for the detection of c-Fos protein, used as an indicator of brain activation.

URB602 alone did not change Fos expression in the brain nuclei under evaluation. When administered 3 hours after NTG, URB602 reduced NTG-induced Fos expression in all the cerebral areas that were examined, with a significant effect in nucleus trigeminalis caudalis and ventrolateral column of periaqueductal grey.

The inhibition of MAGL activity, with the theoretical increase of central content of 2-AG, may modulate the activation of structures involved in pain perception and pain integration in an animal model specific for migraine.

P 8/10

NOVEL ANTISENSE AND PROTEIN CODING GENE VARIATIONS IDENTIFIED BY NGS IN PARKINSON'S DISEASE

Valente Marialuisa[1], Zangaglia Roberta[2], Gagliardi Stella[1], Ricca Ivana[1], Zucca Susanna[1], Blandini Fabio[3], Pacchetti Claudio[2], Cereda Cristina[1]

[1]1. Laboratory of Experimental Neurobiology, C. Mondino National Institute of Neurological Foundation ~ Pavia ~ Italy, [2]3. Parkinson's Disease and Movement Disorders Unit, C. Mondino National Institute of Neurological Foundation ~ Pavia ~ Italy, [3]Center for Research in Neurodegenerative Diseases, C. Mondino National Institute of Neurological Foundation ~ Pavia ~ Italy

The introduction of deep sequencing technologies has revolutionized genetic studies, prompting the development of innovative theories for Parkinson's Disease (PD) pathogenesis. In addition to canonical protein genes associated with PD, potentially relevant antisense genes (AS) have been recently discovered, for example the PINK1 antisense gene (PINK1-AS) (Chiba et al., 2009). This new class of genes transcribed in endogenous RNA molecules of natural antisense transcripts (NATs) that exhibit partial or complete complementarity to mRNAs. NATs contribute to the regulation of sense genes and molecular functions at various levels. Variations in AS genes may interfere with protein gene regulation, influencing PD phenotype expression.

Identification of new genomic variants in either protein and AS genes in a cohort of PD patients.

Next Generation Sequencing analysis has been performed in a cohort of 65 Italian PD patients. True Seq Custom Amplicon platform was composed by PARK2, PINK1, DJ-1, LRRK2, SNCA, UCHL1, EIF4G1, ATP13A2, VPS35 and GBA genes.

Regarding protein genes, the data analysis showed the presence of new non-synonymous mutations in PARK2, LRRK2, PINK1 and ATP13A2. For the mutations in PARK2 (p.W447G, p.R191Q), LRRK2 (p.I178F, p.L2425V) and PINK1 (p.A124V) the in silico prediction (PolyPhen2 and Mutation Tester) supported its pathological implication, while the mutation in ATP13A2 (p.N1091S) resulted neutral. The most interesting data concerned a new AS gene: we found a not yet described SNCA-AS, aside from PINK1-AS. Moreover, several mutations were detected in the SNCA-AS and PINK1-AS, whose functional relevance is currently under investigation.

Our NGS data indicated variations in either protein and AS genes, opening intriguing perspectives in PD genetics. Further studies will be needed to investigate the functional role of AS gene variations, but it is clear that the classic genotype-phenotype vision may no longer be able to explain the complexity of PD molecular mechanisms.

P 9/10

ALTERATIONS OF SYNAPTIC MECHANISMS AND EXCESSIVE GLUTAMATE RELEASE IN THE SPINAL CORD OF SOD1G93A MICE

Bonifacino Tiziana[1], Milanese Marco[1], Musazzi Laura[2], Gallia Elena[1], Cattaneo Luca[1], Treccani Giulia[2], Onofri Franco[3], Popoli Maurizio[2], Bonanno Giambattista[1]

[1]Dept. of Pharmacy, Unit of Pharmacology and Toxicology, University of Genoa, Italy ~ Genoa ~ Italy, [2]Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Italy ~ Milan ~ Italy, [3]Dept. of Experimental Medicine, Unit of Human Physiology, University of Genoa, Italy ~ Genoa ~ Italy

Glutamate(Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS) and reduced astrocytic uptake was suggested as a cause. On the basis of our studies, we have proposed that abnormal release may represent another source for excessive extracellular Glu.

The aim of this study is to investigate at the synaptic level what mechanisms support the excessive Glu exocytosis.

Animals used are SOD1G93A mice expressing high copy number of mutant human SOD1 with a Gly93Ala substitution and SOD1 as controls.
For release experiments, synaptosomes were purified from SOD1 and SOD1G93A spinal cord by homogenization and separation on discontinuous Percoll® gradients. The release of [3H]-D-Aspartate was studied in superfusion.
Ca2+ concentration was measured by fluorometric analysis.
Western blots were performed according to Laemmli et al. (1970) and confocal microscopy according to Stigliani et al.(2006).

Studies were performed at the early and the late phase of the pathology (4 and 17 weeks of life, respectively). We measured Glu release and the results showed that both the spontaneous and the stimulus-evoked exocytotic release of Glu were increased in SOD1G93A mice at both stages of life. We also measured the expression/activation state of a number of pre-synaptic proteins involved in neurotransmitter release (SNAP-25, stx-1A, VAMP-2, synaptophysin, munch-18, munch-13, rab2A, synaptotagmin, complexin 1/2, NSF, α/β snap, dynamin, actin and myosin) by confocal microscopy and western blot experiments. Few of them were found modified and only synaptotagmin and actin resulted over-expressed in both 4 and 17 week old SOD1G93A mice. Increased pre-synaptic Ca2+ levels, over-activation of calcium/calmodulin-dependent kinase-II and ERK/MAP kinases correlates with hyper-phosphorylation of synapsin-I at both early and late stages of disease. In line with these findings, release experiments showed that the excessive Glu exocytosis was accompanied by the increase of the readily releasable pool of vesicles. Supporting the role of the above protein phosphorylation cascade, the excessive glutamate release was prevented by blocking synapsin-I phosphorylation, using specific antibodies.

Our results highlight that aberrant glutamate exocytosis is present in the spinal cord of late stage SOD1G93A mice, an event accompanied by marked changes of specific pre-synaptic molecular mechanisms. The same synaptic alterations are also present in 4 weeks old SOD1G93A mice,

suggesting that they represent a key feature in the early phase of experimental ALS and play a pivotal role in the development of the disease.

P 10/10

THE TUMOR SUPPRESSOR NF2/MERLIN INDUCES SCHWANN CELL CHANGES THROUGH HIPPO-DEPENDENT MECHANISMS

Melfi Simona, Colciago Alessandra, Bonalume Veronica, Giannotti Giuseppe, Caffino Lucia, Fumagalli Fabio, Magnaghi Valerio

Department of Pharmacological and Bimolecular Sciences - University of Milan ~ Milan ~ Italy

Neurofibromin type 2 gene (Nf2) encoding the tumor suppressor protein merlin, a cytoskeleton-associated protein belonging to the ERM (ezrin-radixin-moesin) family, is mutated in an autosomal dominant multiple syndrome called neurofibromatosis type 2. Nf2/merlin inactivation causes a protein loss and leads to Schwann cell (SC) transformation into a form of benign tumor called schwannoma. Furthermore, merlin is involved in different intracellular signalling pathway, such as Hippo and MAPK/ERK. Moreover, Hippo signalling seems to be involved also in changes in SC polarity, migration and proliferation, although the correlation between Nf2/merlin and Hippo should be further investigated. Recently, some challenges, even environmental, have been indicated as pathogenic events regulating neurofibromas growth. Indeed, the exposure to electromagnetic fields (EMF) causes changes in cell migration and cytoskeleton reorganization. Epidemiologic studies show a consistent risk linking the EMF exposure to the onset of neurodegenerative diseases and tumors, including schwannomas.

The aim of this study was to investigate whether changes in Nf2/merlin levels may regulate cellular and biological features, such as morphology, viability, proliferation and also, myelinating capability in SCs following high-frequency EMFs exposure.

EMFs, proliferation and migration assays, chemotaxis analysis, western blot analysis, PCR array, qRT-PCR analysis, immunofluorescence and confocal microscopy.

In our study we found a significant decrease in Nf2 mRNA and protein levels after EMF exposure. Cell proliferation and migration were significantly increased in exposed cells ($P<0.05$). Contemporarily, SCs rise their migratory capability and responsivity to chemotactic agents. Moreover, the expression levels of characteristic myelin proteins, P0 and PMP22, significantly dropped down at all time points considered ($P<0.05$). PCR array shows that some genes, downstream or upstream Nf2, were down-regulated. For instance, proteins Amotl-2, Crb1, 2 and 3, and also Yap1 (the last effector of Hippo cascade) resulted changed. Furthermore, Yap1 was localized mostly in the cytoplasm rather than in the nucleus. We also found an early activation of the MAPK/ERK pathway.

We found that in SCs exposed to the EMFs the oncosuppressor Nf2/merlin is decreased, in turn regulating the cell phenotype toward a proliferative/migrating condition. The MAPK/ERK signalling, which is mainly involved in cell proliferation, is activated. Moreover, the Hippo intracellular signalling is activated. We suggest that the mechanisms involved in EMF-induced SCs transformation are dependent by ERK and Hippo pathways. Therefore, we hypothesize that, when SCs are changed by the EMF exposure, the risk to develop a neurofibroma may increase.

P 11/10

A NEW CLINICALLY RELEVANT MURINE MODEL OF SPINAL CORD INJURY MIMICKING THE PATHOLOGICAL FEATURES OF COMPLETE PARALYSIS IN HUMANS

Vacca Valentina, Pavone Flaminia, Luvisetto Siro, Marinelli Sara

IRCCS Fondazione Santa Lucia, Roma, Italy; CNR - Institute of Cell Biology and Neurobiology (IBCN) ~ Roma ~ Italy

Animal models of spinal cord injury (SCI) try to mimic clinical situations; however, variation in biological processes among species or induced by different experimental designs, have contributed to difficulties in translating experimental findings in animals to the human condition. The actual animal models are far from the reality: i) spinal transection, widely used to assess regeneration but clinically irrelevant, ii) laminectomy before the spinal cord contusion, which reduces the damage and completely eliminates the compression and destruction of bone, or iii) a moderate contusion that in mice does not produce a permanent paralysis.

In this study we present a new mouse model of spinal trauma that shows: (1) total absence of recovery; (2) similar features in pathological nature of the human spinal lesion and, (3) acute and chronic changes in the spinal architecture. Moreover, we propose a description of a device setting to recreate the experimental paradigm.

The SCI was performed in male and female CD1 mice, anesthetized, mounted on a stereotaxic apparatus with spinal adaptors and connected to a precision impactor device. Functional recovery was evaluated by tail flick and Basso Mouse Scale score. Histological features of severe injury were analyzed by immunofluorescence (IF) staining and western blot, both in proximal and in distal area to the lesion, 7 and 30 days after trauma.

Sex-related differences exist in CD1 mice subjected to a severe impact in recovery of both motor function and thermal sensitivity. Different degrees of impact produce different behavioral responses in both sexes (mild vs moderate; mild and moderate vs severe SCI). After a severe trauma male mice gradually recover while females are permanently paralyzed and insensitive. On the other hand, female mice subjected to mild and moderate impact, spontaneously recover.
IF confocal images show how the extent and type of physical damage reflect the ensuing level of functional disruption in female mice, characterizing alterations after injury in the physical structure of spinal cord elements, such as lesion dimension, astrogliosis and cell death.

In conclusion, we developed a new model of SCI in mice that shows long-lasting functional deficits and histological features similar to human pathology. This model can offer a sensitive, reliable, and clinically relevant model for assessing therapeutic interventions for traumatic spinal cord injuries.

P 12/10

HYDROGEN SULPHIDE: A GLIAL FACTOR ENDOGENOUSLY OVERPRODUCED IN AMYOTROPHIC LATERAL SCLEROSIS THAT ENHANCES MOTOR NEURON DEATH

Spalloni Alida[1], Greco Viviana[1], Guatteo Ezia[1], Mercuri Nicola B[2], Urbani Andrea[2], Longone Patrizia[1]

[1]IRCCS-Fondazione Santa Lucia ~ Rome ~ Italy, [2]University of Rome "Tor Vergata" ~ Italy

In a recent study performed in Amyotrophic Lateral Sclerosis (ALS) sporadic patients and in a familial ALS (fALS) mouse model, the classical SOD1G93A mouse, we have found toxic liquoral levels of hydrogen sulphide (H2S) in the patients, higher H2S content in the brain tissues of the fALS mouse, and in the media from SOD1G93A primary mixed spinal cord cultures (Davoli et al., 2015). In the same work we have described an increased toxicity of H2S toward motor neurons (compared to GABAergic neurons) and an increased Ca2+ concentration in spinal cord neurons following H2S treatment, using a H2S analogous NaHS. We have also determined that glial cells release H2S. Since H2S, at toxic levels, is a well known inhibitor of complex IV (cytochrome c oxidase) of the mitochondrial respiratory chain (Modis et al., 2014).

We asked whether the mitochondrial fault described in ALS is related to the H2S metabolism impairment? In addition, we are exploring the toxic pathways through which H2S triggers motor neuron death, with particular care to the pathways associated to the astrocytes-motorneuron cross-talk, going to see if mediators such as Bax are involved.

To address these issues we have performed proteomics and metabonomics analysis on purified neuronal mitochondria derived from the fALS mouse, and analysed toxic pathways (i.e. the Bax pro-apoptotic pathway) on mixed spinal cord cultures.

With this analysis we are trying to determine the energetic mitochondrial turnover by profiling mitochondrial H2S metabolism. Moreover we are aiming at determine the noxious pathways involved in the H2S-induced motor neuron death.

We introduce H2S as a new player to the cohort of pro-inflammatory/degenerative factors that could be involved in the aetiology of ALS.

P 13/10

TREATMENT-RESISTANT PSYCHOSIS IN PATIENTS WITH AUTOIMMUNE THYROIDITIS: A CASE SERIES

Traccis Francesco[1], Baggiani Gioia[1], Loviselli Andrea[2], Bocchetta Alberto[1]

[1]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]Department of Medical Sciences "M. Aresu", University of Cagliari ~ Cagliari ~ Italy

An encephalopathy of presumed autoimmune origin characterized by high titers of antithyroid peroxdase antibodies (AbTPO) has been described. The term Hashimoto's encephalopathy (HE) has also been proposed. The clinical presentation may vary from seizures to stroke-like episodes, cognitive decline, and neuropsychiatric symptoms. The condition is considered an association of an uncommon autoimmune encephalopathy with a common autoimmune thyroid disease.

In 2007 we reported a case of treatment-refractory affective psychosis with autoimmune thyroiditis and started to collect any additional suggestive case of HE. Our aims were to challenge the hypothesis of the rarity of HE and to describe additional cases.

We reviewed clinical charts of patients visited at our psychopharmacology outpatient unit since 2007 and selected those whose AbTPO titer had exceeded by 50 times the upper end of the normal range. Then, we collected their clinical history and analyzed any laboratory test (including markers of inflammation and vasculitis, and nailfold videocapillaroscopy) and neuroimaging potentially related to HE (in particular NMRI and SPECT).

We retrieved 14 cases of suspected HE. Given the nature of our unit, the cases principally regarded patients with a psychiatric presentation. The majority had a presentation of affective psychosis that responded poorly to common treatments and required combined medication.

HE with psychiatric presentation may be more common than previously believed. The links between the clinical pictures, thyroid disease, auto-antibody pattern and brain pathology await further clarification. Similar patterns of SPECT and nailfold videocapillaroscopy have been found in immunomediated diseases presenting with neuropsychiatric symptoms, such as systemic lupus erythematosus, as the probable expression of peripheral and brain vasculitis. Whatever the mechanism, HE should be considered in the differential diagnosis of atypical psychoses. In our experience, treatment-refractory affective psychoses with autoimmune thyroiditis and brain perfusion abnormalities, appear to benefit from lithium combination with antipsychotics. Interestingly, lithium is under investigation in various models of autoimmune disorders among other inhibitors of glycogen synthase kinase-3, a crucial regulator of the balance between pro- and anti-inflammatory cytokine production in both the periphery and the central nervous system.

P 14/10

A NEW TOOL FOR STORING AND INTERROGATING GENOTYPE-PHENOTYPE CORRELATIONS TO STUDY BIPOLAR DISORDER

Melis Carla[2], Rodriguez--Tomé Patricia[3], Ardau Raffaella[1], Chillotti Caterina[1], Del Zompo Maria[2], Squassina Alessio[2], Severino Giovanni[2]

[1]Unit of Clinical Pharmacology ~ University-Hospital of Cagliari ~ Italy, [2]Section of Neurosciences and Clinical Pharmacology, Department of Biomedical Sciences ~ University of Cagliari ~ Italy, [3]NURIDEAS S.r.l ~ Cagliari ~ Italy

The Unit of Clinical Pharmacology University-Hospital of Cagliari see patients affected by bipolar disorder, a chronic psychiatric illness characterized by the recurrence of manic and depressive episodes. The phenotypic representation of bipolar disorder is variable, several evidence suggest contributions from genetic and environmental factors. General practitioners and psychiatrists may be able to evaluate the individual patient's clinical course and the therapies impact on it. Several medications are available for the treatment of bipolar disorder, however these need to be monitored in order to guarantee the clinical efficacy, prevent the recurrences, the adverse reactions and ultimately reduce the risk of suicide.

The aim of our project is to develop a database to integrate the clinical information collected from patients followed at the Unit of Clinical Pharmacology University-Hospital of Cagliari and with the corresponding genetic data obtained from the biobank located in the laboratory of the Section of Neurosciences and Clinical Pharmacology of the Department of Biomedical Sciences of University of Cagliari.

We have developed the database using the MySQL RDBMS, on an Ubuntu server. The interface is developed in PHP using Symfony framework. The server is installed in a protected environment providing secure access to the users with different privilege levels for the access of sensitive data, therefore ensuring the privacy of the patients.

Our database must provide: 1) access and tools to doctors interviewing the patients, 2) ability to retrieve and analyze data for doctors carrying out clinical research, 3) a way to manage experimental results for biologists in the laboratory 4) data retrieval and analysis tools for biostatisticians.

In conclusion, this database will be a useful tool for doctors and researchers to investigate the impact of the drug therapies, to maximize therapeutic benefit and to minimize adverse events at clinical and molecular level.

P 15/10

SUMOYLATION OF NCX3 BY SUMO1 TAKES PART IN THE BRAIN NEUROPROTECTION INDUCED BY ISCHEMIC PRECONDITIONING

Cuomo Ornella[1], Pignataro Giuseppe[1], Sirabella Rossana[1], Anzilotti Serenella[2], Molinaro Pasquale[1], Scorziello Antonella[1], Savoia Claudia[1], Sisalli Maria Josè[1], Di Renzo Gianfranco[1], Annunziato Lucio[1]

[1]Division of Pharmacology, Department of Neuroscience, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II" University of Naples ~ Naples ~ Italy, [2]SDN IRCCS ~ Naples ~ Italy

The small ubiquitin-like modifier (SUMO) is a post-translational protein modification mechanism activated by several stresses that has been investigated in experimental models of cerebral ischemia only in the last years. Convincing evidences showed that sumoylation can confer neuroprotection against stressful stimuli by regulating the function and the fate of proteins involved in stress signalling pathways. Recently, it has been shown that sumoylation enzymes and substrates are expressed not only in the cytoplasmic and nuclear compartments, but also at the plasma membrane level. Among the numerous plasmamembrane proteins controlling ionic homeostasis during cerebral ischemia, we demonstrated that NCX3 exerts a protective role during cerebral ischemia.

The aim of the present study was to evaluate whether sumoylation of NCX3 by SUMO1 takes part to brain neuroprotection induced by ischemic preconditioning. We analyzed: (1) SUMO1 conjugation pattern after ischemia and ischemic preconditioning; (2) the effect of SUMO1 knocking down on the ischemic damage after tMCAO; (3) the effect of SUMO1 knocking down on the neuroprotection mediated by ischemic preconditioning; (4) the possible interaction between SUMO1 and NCX3 and the molecular determinants of NCX3 sequence responsible for sumoylation.

Focal brain ischemia and ischemic preconditioning were experimentally induced in adult male rats by subjecting them to different protocols of middle cerebral artery occlusion and reperfusion (MCAO). SUMOylation was evaluated by Western Blot analysis and immunohistochemistry in temporoparietal cortex and striatum of rats subjected to tMCAO or ischemic preconditioning. SUMO1 and NCX3 interaction was analyzed by site-directed mutagenesis and immunoprecipitation assay in BHK cells transfected with different NCX3 mutants.

Collectively, our results showed that (1) SUMO1 knocking-down worsened the ischemic damage and prevented the protective effect of ischemic preconditioning, (2) more important, SUMO1 binds NCX3 and SUMO1 silencing results in the increase of NCX3 degradation, and (3) NCX3 sumoylation takes part to SUMO1 protective role during ischemic preconditioning.

Overall our results suggest that NCX3 sumoylation may represent a new potential target to enhance the brain neuroprotection induced by ischemic preconditioning.

P 16/10

FOXG1 OVEREXPRESSION IN NEOCORTICAL NEURONS: A VALUABLE TOOL TO DISSECT MECHANISMS UNDERLYING WEST SYNDROME VARIANTS LINKED TO FOXG1 GENE DUPLICATION

Pinzan Moira[1], Do Duc Minh[1], Chiola Simone[1], Provenzano Giovanni[2], Bozzi Yuri[2], Mallamaci Antonello[1]

[1]SISSA ~ Trieste ~ Italy, [2]Centre for Integrative Biology (CIBIO) ~ Trento ~ Italy

West Syndrome (WS) occurs in circa 1 every 3,500 infants. It includes infantile-onset spasms, prominent interictal EEG abnormalities and impaired psychomotor development. Spasms and EEG abnormalities often disappear before 3 years of age. However, the majority of patients will develop other types of epileptic syndromes. The etiology of the syndrome is complex and includes genetic as well as not-genetic factors. In up to 5% of cases, WS is associated to microduplications including FOXG1, a key transcription factor gene mastering early telencephalic development.

Main aim of our work was to clarify molecular mechanisms linking increased Foxg1 gene dosage to CNS hyperexcitability.

We addressed this issue in vivo and in vitro. We generated conditional Foxg1-gain-of-function (GOF) mouse mutants, by integrated use of classical transgenesis, knocking-in and TetOFF technology. We took advantage of primary cultures of cortico-cerebral neurons, engineered by recombinant lentiviruses and TetON technology. Lastly, as a complementary approach, we cotransplanted engineered and control pallial precursors, labelled by distinct fluoroproteins, into perinatal wild type brains.

We scored Foxg1-GOF mutants for transgene activity, which was unexpectedly confined to postmitotic neocortical neurons. Histological inspection of mutant brains did not show any gross abnormalities. However, neocortical laminar specification was perturbed by an apparent spreading of layer V identities to adjacent layers. Excitability of these mutants, probed by subconvulsant doses of kainic acid and behaviourial profiling, was increased in a robust and reproducible way. In particular, the immediate-early gene c-fos was upregulated, strongly in the hippocampus and - to lesser extent - within the mutant neocortex. Looking for basic mechanisms underlying hyperexcitability, we discovered three key issues putatively contributing to it. Foxg1-GOF neurons displayed a far enriched and mature dendritic tree, possibly conveying afferences from a wider excitatory basin. The number of grey matter astrocytes was significantly reduced. Neuronal expression levels of selected neurotransmitter receptors were unbalanced.

Summarizing, the etiopathogenesis of Foxg1-linked WS is complex and includes both strictly "neuronal" factors and "not-neuronal" anomalies. It will be of paramount importance to dissect their fine molecular and temporal articulation, in order to define possible therapeutic windows, if any, to exploit for rationale treatment of small patients.

P 17/10

SUPEROXIDE DISMUTASE 1: A NEW FUNCTION IN THE NUCLEAR COMPARTMENT

Pansarasa Orietta[1], Gagliardi Stella[1], Dell''Orco Michela[1], Bordoni Matteo[2], La Salvia Sabrina[3], Diamanti Luca[4], Maffioli Elisa[5], Tedeschi Gabriella[5], Cereda Cristina[1]

[1]Laboratory of Experimental Neurobiology, C. Mondino National Institute of Neurology Foundation, IRCCS, ~ Pavia ~ Italy, [2]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia ~ Pavia ~ Italy, [3]Department of Public Health, Neuroscience, Experimental and Forensic Medicine, University of Pavia, ~ Pavia ~ Italy, [4]Department of Brain and Behavioural Science, University of Pavia ~ Pavia ~ Italy, [5]Filarete Foundation ~ Milan ~ Italy

Recent evidences showed that, in PBMCs from sALS patients, there is an over-expression of SOD1 mRNA (Gagliardi et al, 2010), which is in contrast with the unchanged cytoplasmic level of the protein (Cova et al, 2006). This discrepancy could be explained considering a re-localization of the "missing" protein in the nucleus. Moreover, in PBMCs of sALS patients, SOD1 translocates from the cytoplasm to the nuclear compartment in stressful conditions (Cereda et al, 2013).

The exact role of SOD1 in the nuclear compartment remains a critical issue to clarify. Thus, we aim to investigate whether and how nuclear SOD1 could act against oxidative stress both in neuroblastoma cell line SHSY5Y, a cellular model of neurodegeneration, and in PBMCs of sALS patients.

SOD1 localization in SHSY5Y and in sALS patients was investigated by both WB and immunofluorescence. By means Mass Spectrometry (MS) we searched for the modifications allowing for SOD1 translocation and by immunoprecipitation (IP) we identify the binding protein involved in regulation of SOD1 localization. Finally, by Comet assay we studied if nuclear SOD1 could exert a protective role towards DNA damage.

First, we confirmed that under oxidative stress SOD1 re-locates into the nucleus; SOD1 levels decreases after 30 min of 1mM H2O2 treatment, and rescues at T60; probably as a consequence of new protein synthesis. MS data highlight that SOD1 nuclear re-localization is prompted by phosphorylation of both serine and threonine at T60. Moreover, the kinase enzyme Chk2 seems to play a critical role in the regulation of SOD1 localization. Comet assay revealed that SOD1-NLS cells showed no comets, indicating that, when SOD1 is located into the nucleus, minor or none DNA fragmentation occurred. Finally, an increase in histone H3 acetylation at both T30 and T60, suggested us a possible involvement of nuclear SOD1 in gene transcription.

In response to oxidative stress, we demonstrated that SOD1 re-locates at nuclear level, where it plays new functions. We reported an involvement of nuclear SOD1 in order to provide resistance against oxidative DNA damage, and a role in the regulation of gene transcription.

P 18/10

SIMILARITIES AND DIFFERENCES BETWEEN ALS AND IBM

Pansarasa Orietta[1], Cortese Andrea[2], Bordoni Matteo[3], Polveraccio Francesca[1], Drufuca Lorenzo[1], Buratti Emanuele[4], Cereda Cristina[1]

[1]Laboratory of Experimental Neurobiology, C. Mondino National Institute of Neurology Foundation, IRCCS, ~ Pavia ~ Italy, [2]General Neurology Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, ~ Pavia ~ Italy, [3]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia ~ Pavia ~ Italy, [4]International Centre for Genetic Engineering and Biotechnology (ICGEB) ~ Trieste ~ Italy

TDP43 is a major component of the inclusions that characterize FTD and ALS pathology, and sporadic inclusion body myositis (sIBM) muscle pathology. Recent findings (D'Agostino et al, 2011; Hernandez Lain et al, 2011), have strengthened the link between sIBM and neurodegenerative disorders, also supported by age of disease onset; identification of neurodegeneration-characteristic proteins in ubiquitinated inclusions of sIBM muscle; and identification of VCP mutations as a cause of ALS, and a complex phenotype which comprises an hereditary form of IBM associated with FTD.

We aim to investigate similarities and/or differences in PBMCs of sALS, sIBM and healthy controls (CTRL) in the expression of the main RNA binding protein (TDP43, FUS and hnRNPA2/B1).

We used PBMCs obtained from sALS, sIBM and CTRL. Using soluble and insoluble total protein fractions as well as the nuclear and cytoplasm compartments we analyzed TDP43, FUS and hnRNPA2/B1 expression levels by WB and immunofluorescence.

In total soluble fraction we observed a significant increase ($p<0.05$) in the expression of TDP43 in sIBM compared to CTRL; FUS expression levels did not change. When considering hnRNPA2/B1 expression levels, we reported a trend of decrease in both sALS and sIBM compared to CTRL. In total insoluble fraction TDP43 expression levels tend to increase in sALS and sIBM compared to CTRL; FUS decrease significantly ($p<0.001$) in sALS versus CTRL, moreover we did not detect FUS levels in sIBM. Similarly, in CTRL, sALS and sIBM, the expression of hnRNPA2/B1 was not detectable.
Regarding the subcellular localization (nuclear or cytoplasm) of these proteins, we reported significant changes only for hnRNPA2/B1 distribution. In particular, a significant increase in nuclear and cytoplasm compartment in sIBM compared to both CTRL and sALS could be detected.
Immunofluorescence results suggested a different localization of all the examined RNA binding proteins between sIBM and CTRL as well as between sIBM and sALS. In particular, in sIBM we observed the presence of cytoplasm aggregates of TDP43 and hnRNP which seem to colocalize, even if hnRNPA2/B1 have also a nuclear distribution. In sALS, we reported that TDP43, FUS and hnRNP have a more diffuse distribution among nucleus and cytoplasm.

We report that PBMCs could be intriguing as a cellular model to study sALS and sIBM. The different features we observed further consolidate the idea of a link between sIBM and neurodegenerative disorders. Moreover, our data seem to suggest that hnRNPA2/B1 could represent a differentiative element between the two pathologies.

P 19/10

SUPRAMODAL MIRROR AGNOSIA IN PATIENTS WITH PERIVENTICULAR LEUKOMALACIA

Castaldi Elisa[1], Tinelli Francesca[2], Cicchini Guido Marco[3], Biagi Laura[2], Morrone Maria Concetta[1]

[1]Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa ~ Pisa ~ Italy, [2]IRCCS Stella Maris Scientific Institute ~ Pisa ~ Italy, [3]CNR, Neuroscience Institute ~ Pisa ~ Italy

Periventricular leukomalacia (PVL) is characterized by focal white matter necrosis often observed in preterm infants. PVL is frequently associated with motor impairment and with visual deficits affecting primary stages of visual processes as well as higher visual cognitive abilities.

We describe five PVL subjects, with normal IQ and visual contrast sensitivity, showing orientation perception deficits in both the haptic and visual domains.

The subjects were asked to compare the orientation of two stimuli presented simultaneously or sequentially, using both a matching and a 2AFC orientation-discrimination procedure. The visual stimuli were oriented gratings or bars or collinear short lines embedded in a random pattern. The haptic stimuli consisted in a pair of bars. One patient was also examined by fMRI, where second-order visual stimuli were presented to peripheral view.

PVL patients performed at chance in discriminating the oblique orientation both for visual and haptic stimuli. Moreover when asked to reproduce the oblique orientation, they often oriented the stimulus as its symmetric mirror. The deficit cannot be explained by simultaneous agnosia nor by impaired visual memory: it also occurs for sequential presentations, but not for horizontal or vertical stimuli. In control subjects, area V3A was orientation-selective for the second-order pattern, while the PVL patient showed an anomalous selectivity for the oblique orientation that was consistently confused with the mirror image.

These findings show that PVL can affect a specific network involved with supramodal perception of mirror orientation.

P 20/10

CAN LOSS OF HETEROZYGOSITY EXPLAIN CEREBRAL CAVERNOUS MALFORMATIONS' DEVELOPMENT IN PATIENTS WITH NO CCM GENES GERMLINE MUTATIONS?

Scimone Concetta[2], Alafaci Concetta[1], Ruggeri Alessia[2], Donato Luigi[2], Rinaldi Carmela[2], D''Angelo Rosalia[2], Sidoti Antonina[2]

[1]Department of Neurosciences, University of Messina ~ Messina ~ Italy, [2]Department of Biomedical Sciences and Morphological and Functional Images, Division of Biology and Genetics, University of Messina ~ Messina ~ Italy

Cerebral Cavernous Malformations (CCMs, OMIM 116860) are vascular lesions that affect cerebral microvascular endothelial cells; brain parenchyma is not involved. Most common clinical manifestations are intracerebral hemorrhage due to alterations at endothelial cells junctions, and seizures, headaches and focal neurological deficits as consequence of lesions' localization. CCMs incidence is estimated to be 0,1% - 0,5% wordwide and they can occur both in familial form and in sporadic one. Familial forms usually appear with multiple lesions since pediatric age and are inherited with an autosomal dominant pattern, linked to germ-line mutations at the three loci CCM1/KRIT1, CCM2/MGC4607 and CCM3/PDCD10; they are characterized by neuroradiological and clinical incomplete penetrance and variable expressivity. Sporadic forms arise between 3rd and 50th decades of life, often with single lesion. About mutation's rate, among familial cases 56%, 16% and 17% are linked to CCM1, CCM2 and CCM3, respectively; while, among sporadic ones these percentages amount to 33%, 10% and 14%.

Absence of germline mutations in 11% and 43% of familial and sporadic cases respectively can be explained by several hypothesis. Among of these, the presence of somatic mutations at the CCM genes, the possible involvement of a still unknown CCM4 gene, epigenetics factors.

A group of patients affected by sporadic forms, undergone surgery and negative for CCM genes germline mutations were involved. Germline/somatic comparison was performed on DNA extracted by blood and pathological endothelial cells, respectively. Endothelial cells were isolated from biopsies by Fluorescence Activated Cell Sorting (FACS) using anti-CD31 antibody. All CCM genes coding exons were amplified by PCR and sequenced at 3500 Genetic Analyzer (Applied Biosystems).

Analysis is not yet complete. To date, it seems there are no differences between germline and somatic DNA.

If absence of somatic mutations will be confirmed for all considered patients, loss of heterozygosity, still assessed as one of the most important pathogenetic mechanism, will be further argued. Development of these lesions, characterized by a complex molecular pathogenesis, indeed, can be explained by more approaches such as transeterozigosity condition and modifier genes involvment.

P 21/10

ROLE OF MELOXICAM ON GLUTAMATE AND GABA CYCLE MODIFICATIONS IN RAT ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES EXPOSED TO OXYGEN-GLUCOSE DEPRIVATION

Landucci Elisa[1], Llorente Irene Lorenzo[2], Gerace Elisabetta[1], Fernandez--Lopez Arsenio[2], Pellegrini--Giampietro Domenico[1]

[1]Department of Health Sciences, Section of Clinical pharmacology and Oncology, University of Florence ~ Florence ~ Italy, [2]Area de Biologia Celular, Istituto de Biomedicina, University of Leon ~ Leon ~ Spain

Stroke causes brain dysfunction and neuronal death, and the lack of effective therapies heightens the need for new therapeutic targets. Meloxicam is a non-steroidal anti-inflammatory drug which has been reported to lessen the ischemic transcriptional effects in some of the glutamatergic system genes, as well as to decrease the infarct volume in in vivo assays.

The aim of the study was to evaluate the effects of meloxicam in glutamate and GABA cycle in an in vitro model of cerebral ischemia.

In this study, the neuroprotective effects of meloxicam were evaluated in rat organotypic hippocampal slices exposed to oxygen-glucose deprivation (OGD). Transcript levels of glutamatergic and GABAergic system genes were measured by real time PCR and proteins were evaluated by Western blotting.

When present in the incubation medium, meloxicam reduced CA1 injury induced by OGD. Meloxicam was able to selectively increase or decrease the OGD-induced changes in the expression of a number of genes, including: vescicular glutamate trasporters (VGLUT1, VGLUT2, GLAST-1A, GLT-1, and EAAC-1), glutamate (NMDA and AMPA) and GABAA receptor subunits, but did not modify membrane glutamate transporters.

In conclusion, our study suggest that the neuroprotective role of meloxicam could be due to a modification in the balance of glutamatergic and GABAergic receptor subunits, leading to a different stoichiometry of NMDA or AMPA receptors.

P 22/10

IMPAIRMENT OF CORTICO-STRIATAL GLUTAMATERGIC SYNAPSES IN TWO MOUSE MODELS OF HUNTINGTON'S DISEASE (HD)

Talpo Francesca[2], Zuccato Chiara[3], Cattaneo Elena[3], Sancini Giulio[4], Toselli Mauro[1], Biella Gerardo[1]

[1]Dept Biology and Biotechnology, University of Pavia ~ Pavia ~ Italy, [2]~ Pavia ~ Italy, [3]Dept of Biosciences, University of Milano ~ Milano ~ Italy, [4]Dept Health Sciences, University of Milano-Bicocca ~ Milano ~ Italy

HD is a neurodegenerative disorder mainly due to basal ganglia and cortico-striatal circuits impairments. HD causes a widespread loss of cortical pyramidal neurons (Pyr) and striatal medium-sized spiny neurons (MSNs) and the deconstruction of the Pyr/MSNs glutamatergic synapses. MSNs are modulated by striatal fast-spiking interneurons (FS-INs) through feed-forward inhibition.

Despite the central role of the striatal FS-INs, the impact of HD mutation onto the glutamatergic Pyr/FS-INs connectivity has not yet been investigated

Here we performed whole-cell patch-clamp experiments on brain slices obtained from WT and R6/2 HD mice (aged 11-12 weeks) to study the impairments of the following cortico-striatal glutamatergic synapses i) Pyr/MSNs and ii) Pyr/FS-INs.

In WT mice, spontaneous excitatory currents (sEPSCs) recorded from FS-INs show a higher frequency and faster kinetic parameters compared to sEPSCs recorded from MSNs. In R6/2 mice the frequency of sEPSCs from both MSNs and FS-INs was significantly reduced compared to WT and this reduction was stronger in FS-INs. Furthermore a reduction in the frequency of mini-EPSCs in R6/2 vs WT mice has also been observed. These results have partially been replicated also in Zq175 HD mice

This study shows that both Pyr/MSNs and Pyr/FS-INs cortico-striatal synapses are compromised in HD. These patho-physiological readouts of the HD could be used to define in an vitro or in vivo model the therapeutic effects of newly developed compound for HD.

P 23/10

PRION PROTEIN INFLUENCES ALPHA-SYNUCLEIN AGGREGATES SPREADING IN MICE

Masperone Lara, Aulic Suzana, Isopi Elisa, Narkiewicz Joanna, Legname Giuseppe

Area of Neuroscience, S.I.S.S.A. ~ Trieste ~ Italy

Conversion, replication and transmission are unique features of prions in prion diseases. More recently these characteristics have been attributed to other proteins involved in more common forms of neurodegenerative disorders. At the same time, several lines of evidence have implied the involvement of the cellular form of the prion protein (PrPC) in the modulation of the toxicity of A-beta oligomers such as in Alzheimer's disease.

In this work we investigated whether PrPC is also involved in the propagation of alpha-synuclein (alpha-syn) in Parkinson's disease (PD).

Synthetic alpha-syn fibrils were stereotaxically inoculated into the substantia nigra pars compacta of FVB PrP wild-type (WT) and knockout (KO) mice. Immunohistochemical and immunofluorescence analysis was performed to assess the presence of alpha-syn aggregates before and after Proteinase-K (PK) digestion in presence or absence of PrPC. The presence of phosphorylated alpha-syn, as well as the activation of astroglia and microglia was also investigated.

After 9 month post operation, infected mice showed a widespread presence of PK-resistant alpha-syn aggregates, whose expression is greater in FVB WT mice, rather than FVB KO mice. This data is in line with in vitro evidence showing that uptake of alpha-syn amyloids is lower in N2aKO cells compared to controls. Moreover, immunopositive GFAP and Iba1 positive cells were indicative of the development of the neurodegenerative pathology. The loss of dopaminergic neurons is a known feature in PD pathology, as well as the presence of phosphorylated alpha-syn into aggregates. Further work has to be done to investigate the decrease of TH-positive neurons in inoculated mice, and eventually the presence of phosphorylated aggregates in the dopaminergic circuits, as the primary place of formation and maturation of aggregates, before the spreading throughout the brain.

Our findings suggest a role for PrPC in regulating of alpha-syn fibrils uptake and disease progression. Moreover, it stresses a link between the two neurodegeneration-associated proteins and suggests an overlap between prion diseases and PD.

P 24/10

TRAF6 INVOLVEMENT IN PRION DISEASE: A POSSIBLE CROSSTALK AMONG NEURODEGENERATIVE DISEASES

Masperone Lara, Zucchelli Silvia, Legname Giuseppe

Area of Neuroscience, S.I.S.S.A. ~ Trieste ~ Italy

The tumor necrosis factor receptor (TNFR)- associated factors 6 (TRAF6) has been found to be involved in Parkinson's disease as well as in Huntington's disease. In cellular models of these diseases TRAF6 has been shown to bind to disease-associated proteins (DJ-1, α-synuclein and huntingtin) and to promote their atypical ubiquitination leading to accumulation of insoluble forms of ubiquitinated proteins into cytoplasmic aggregates.

In this work we studied the interplay between TRAF6 and cellular prion protein (PrPC), analysing two different isoforms of PrPC: the full-length PrPC (FL-PrPC) and the cytosolic PrPC (cyPrPC).

HEK293T cells were transfected with flagged-TRAF6 (FLAG-T6) and FL PrPC or cyPrPC constructs, or with GFP-T6 and FLAG-PrPC constructs and used to study TRAF6 interaction with PrPC. Experiments were performed in steady state conditions or upon proteasome inhibition with reversible (MG132) and irreversible (Lactacystin) inhibitors. TRAF6 and PrPC co-localization was followed by immunofluorescence analysis. Functional interplay between the two proteins was studied by cellular fractionation experiments and monitoring the accumulation of insoluble aggregates.

Co-immunoprecipitation experiments revealed that TRAF6 interacts with both forms of PrPC (FL-PrPC and cyPrPc) and this interaction is further stimulated in conditions of proteasome inhibition. When co-expressed, FL PrPC and TRAF6 can be found to colocalize in the vicinity of the plasma membrane. Protein colocalization is extended to the cytoplasm when cyPrPC is used. Finally, both FL and cyPrPC are present in the insoluble cellular fraction together with TRAF6 when expressed in HEK cells.

This data show a physical interaction between TRAF6 and PrPC. Accumulation of the two proteins into cellular insoluble fraction indicates a possible functional interplay. Therefore, TRAF6 may play a common role in the regulation of neurodegenerative disease-related proteins, including PrPC.

P 25/10

FUNCTIONAL CHARACTERIZATION OF A NOVEL MUTATION AFFECTING THE FIRST ARGININE IN THE S4 SEGMENT OF KV7.2 CHANNEL CAUSING EARLY-ONSET EPILEPTIC ENCEPHALOPATHY

Miceli Francesco[1], Soldovieri Maria Virginia[2], Ambrosino Paolo[2], Cooper Edward C[3], Taglialatela Maurizio[2]

[1]Department of Neuroscience, Reproductive and Odontostomatological Sciences, University of Naples "Federico II" ~ Naples ~ Italy, [2]Department of Medicine and Health Science, University of Molise ~ Campobasso ~ Italy, [3]Department of Neurology and Department of Neuroscience and Molecular and Human Genetics, Baylor College of Medicine ~ Houston ~ United States

Mutations in KCNQ2 and KCNQ3 genes, encoding for the Kv7.2 and Kv7.3 voltage-gated K+ channel subunits, have been identified in patients with Benign Familial Neonatal Seizures (BFNS), a rare autosomal-dominant epilepsy of the newborn with mostly benign neurodevelopmental outcome. More recently, KCNQ2 mutations have been also described in neonates affected with Early-Onset Epileptic Encephalopathy, a group of devastating epilepsies characterized by refractory seizures and neurodevelopmental delay. Kv7.2 and Kv7.3 channels are mainly expressed in the Central Nervous System where they form homo- or hetero-tetrameric channels underlying a K+ current called M-current which regulates neuronal firing.

Mutagenesis, electrophysiological and molecular modeling techniques have been used to investigate the consequences prompted by a novel mutation neutralizing the first Arg in the S4 segment of Kv7.2 channels (R198Q), identified in three unrelated families with epileptic encephalopathy and later-onset seizures reported into a case registry/database (www.rikee.org).

We introduced the specific mutation in the human KCNQ2 cDNA and studied their functional properties using the whole-cell configuration of the patch-clamp technique upon their transient expression in CHO cells.

Electrophysiological experiments revealed that homomeric Kv7.2 R198Q subunits exhibited an approximately 2-fold increase in maximal current density and a robust leftward shift in activation voltage-dependence of about 30 mV. When expressed with wild-type Kv7.2 and Kv7.3, to mimic the heterozygous genotype of affected patients, the current density was equal to control, but activation was shifted approximately 10 mV to hyperpolarized potentials. These results suggest that this mutation, similar to those affecting the proximal part of S4, induced a gain of function (GOF) effect on Kv7.2 channels. Therefore, in order to attempt to counteract these mutation-induced effects on Kv7.2 currents, we evaluated the effects of pH on currents elicited by heteromeric channels incorporating Kv7.2 R198Q subunits, since Kv7.2/3 currents are inhibited by H+ ions in a voltage-dependent manner. A decrease of pH dose-dependently shifted the voltage-dependence of current activation to the right; at pH 6.4 we observed an almost complete restoration of wild-type voltage-dependence.

We identified a GOF mechanism in Kv7.2 R198Q, a novel mutation that causes epileptic encephalopathy. Furthermore, we found that a decrease in pH significantly reverses the GOF of

the mutant channel, suggesting that drugs causing a moderate acidosis, such as the carbonic anhydrase inhibitor acetazolamide, may potentially be of benefit in this specific subgroup of patients.

P 26/10

KNOCKING OUT THE NA^+/CA^{2+} EXCHANGER NCX3 IMPAIRS OLIGODENDROCYTE LINEAGE RESPONSES, ANTICIPATES THE ONSET, AND INCREASES THE SEVERITY OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Casamassa Antonella[3], Boscia Francesca[3], La Rocca Claudia[1], Matarese Giuseppe[2], Annunziato Lucio[3]

[1]IEOS-CNR ~ Naples ~ Italy, [2]Department of Medicine, School of Medicine , University of Salerno ~ Salerno ~ Italy, [3]Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples ~ Naples ~ Italy

The Na+/Ca2+ exchanger NCX3, recently identified as a myelin membrane component, is involved in regulating intracellular Ca2+ concentration during oligodendrocyte maturation. The importance of this key mediator of sodium and calcium homeostasis was examined in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an animal model of Multiple Sclerosis.

The study objectives included: 1) To assess all inflammatory, neuronal and glial responses after EAE immunization; 2) To provide a clear picture of NCX3 expression and distribution in the white matter spinal cord during EAE course; 3) To establish the role played by NCX3 in EAE severity and progression.

Biochemical and immunohystochemical analyses were performed with the aim to analyze the expression profile of NCX3 protein in the spinal cords of control and EAE mice. Behavioural, morphological and cytometry analyses were performed in NCX3 congenic wild type and in NCX3 knockout mice exposed to EAE with the aim to study the neurological deficits, the demyelination, the axonal loss, the oligodendroglial responses and the number of immune T-cell subsets during the pathology.

Western blotting and quantitative colocalization studies performed in wild-type ncx3+/+ mice at different stages of EAE disease showed that NCX3 protein was intensely upregulated at chronic stage where it was intensely coexpressed by the OPC marker NG2 and the premyelinating marker CNPase. Homozygous mice lacking ncx3 gene (ncx3-/-) not only displayed a reduced diameter of axons and intact myelin ring number but also a dramatic decrease in OPC and pre-myelinating cells in the white matter spinal cord if compared to congenic ncx3+/+ mice at chronic disease stage. Accordingly, homozygous ncx3 -/- and heterozygous ncx3 +/- mutants displayed an anticipated development of EAE disease and increased severity of clinical symptoms. Interestingly, in ncx3-/- mice, the number of immune T-cell subsets, revealed by cytometry analysis at the peak of EAE disease, was not statistically different from those measured in congenic ncx3+/+.

Our findings demonstrate that knocking out NCX3 exchanger impairs oligodendrocyte lineage responses and worsens clinical symptoms in EAE-induced Multiple Sclerosis without involving alterations in immune T-cell population.

P 27/10

RHES INFLUENCES STRIATAL CAMP/PKA-DEPENDENT SIGNALING AND SYNAPTIC PLASTICITY IN A GENDER-SENSITIVE FASHION

Ghiglieri Veronica[1], Napolitano Francesco[2], Pelosi Barbara[3], Pendolino Valentina[4], Giampà Carmela[4], Schepisi Chiara[4], Migliarini Sara[3], Di Maio Anna[5], Vitucci Daniela[5], Mancini Maria[4], Sciamanna Giuseppe[7], Maddaloni Giacomo[3], Errico Francesco[5], Nisticò Robert[6], Pasqualetti Massimo[3], Picconi Barbara[4], Usiello Alessandro[5]

[1]University of Perugia ~ Perugia ~ Italy, [2]University of Naples ~ Naples ~ Italy, [3]University of Pisa ~ Pisa ~ Italy, [4]IRCCS Fondazione Santa Lucia ~ Rome ~ Italy, [5]CEINGE Biotecnologie Avanzate ~ Naples ~ Italy, [6]University of Rome La Sapienza ~ Rome ~ Italy, [7]University of Rome Tor Vergata ~ Rome ~ Italy

The Ras homolog enriched in striatum (Rhes) is a small GTP-binding protein highly expressed throughout the dorsal striatum and nucleus accumbens of rodent brain. Rhes transcription is regulated by thyroid hormones during development and by dopamine (DA) in the adult rat brain. Previous studies indicated that lack of Rhes resulted in sex–sensitive behavioral phenotypes in mutants, but the mechanisms are unknown.

This study is aimed at dissecting the mechanisms of the gender-specific differences associated to Rhes deletion, focusing on striatal cAMP/PKA-dependent signaling and tested the differential motor responses elicited by DA and adenosine agonist/antagonists.

The study was conducted in both male and female Rhes knockout (Rhes KO), generated by crossing Rhes+/loxP-neo offspring with a CMV-Cre deleter strain in a B6D2 genetic background and in and wild-type (WT) controls. To identify the striatal neurons that express Rhes, we performed double in situ-hybridization on striatal and hippocampal sections in WT mice and on human striatal and hippocampal brain samples. By in vitro patch clamp and intracellular recordings we studied synaptic properties and corticostriatal plasticity of medium spiny neurons (MSNs) while extracellular recordings of field excitatory postsynaptic potentials were carried out in hippocampal slices. Western blot analysis was used to determine phosphorylation state of glutamate AMPA receptors as index of cAMP/PKA signaling pathway activity, and protein levels of glutamate, adenosine and estrogen receptors in striatal samples. Double immunofluorescence was employed to characterize striatal DA D1-expressing and A2A/D2-expressing MSNs of direct and indirect striatonigral pathways, respectively.

While Rhes KO male mice, compared to WT mice, had a significant basal increase of cAMP/PKA signaling pathway, the Rhes KO females exhibited a much stronger response of this pathway, selectively under the conditions of dopamine/adenosine-related drug challenge. Corticostriatal LTP defects are exclusively found in A2AR/D2R-expressing MSNs of KO females, compared to KO males, an effect that is abolished by PKA inhibitors, but not by the removal of circulating estrogens. This suggests that the synaptic alterations found in KO females could be triggered by an aberrant A2AR/cAMP/PKA activity, but not due to estrogen-mediated effect. Consistent with increased cAMP signaling, D1R-mediated motor stimulation, haloperidol-induced catalepsy and caffeine-evoked hyper-activity are robustly enhanced in Rhes KO females compared to mutant males.

Overall, our study indicates that Rhes alters the striatal cAMP/PKA signalling cascade differently in male and female mice, orchestrating gender-sensitive alterations in striatal signaling, synaptic plasticity, and behavioral responses.

P 28/10

RHES REGULATES DOPAMINE D2 RECEPTOR TRANSMISSION IN STRIATAL CHOLINERGIC INTERNEURONS

Sciamanna Giuseppe[1], Napolitano Francesco[2], Pelosi Barbara[3], Bonsi Paola[4], Vitucci Daniela[5], Nuzzo Tommaso[5], Punzo Daniela[5], Ghiglieri Veronica[6], Ponterio Giulia[1], Pasqualetti Massimo[3], Pisani Antonio[1], Usiello Alessandro[7]

[1]Department of Systems Medicine, University of Rome "Tor Vergata", ~ Rome ~ Italy, [2]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II" ~ Napoli ~ Italy, [3]Department of Biology, University of Pisa, Via L. Ghini 13, 56126 Pisa, Italy. ~ Pisa ~ Italy, [4]IRCCS Fondazione Santa Lucia, Laboratory of Neurophysiology and Synaptic Plasticity ~ Rome ~ Italy, [5]CEINGE Biotecnologie Avanzate Department of Health and Motor Sciences ~ Napoli ~ Italy, [6]Department of Philosophy and Human Sciences, University of Perugia ~ Perugia ~ Italy, [7]Department of Environmental Sciences, Second University of Naples (SUN) ~ Napoli ~ Italy

Ras homolog enriched in striatum (Rhes) is a small monomeric GTP-binding protein, predominantly localized throughout dopaminoceptive neurons of dorsal striatum and nucleus accumbens. Rhes expression is developmentally regulated by thyroid hormone in rodents and by dopamine (DA) innervation in adult rats. Studies in cell lines have indicated that Rhes, most likely through its binding to Gαi, reduces G-protein coupled receptor (GPCR)-mediated cAMP accumulation. Moreover it has been shown that Rhes also activates mammalian target of rapamycin complex1 (mTORC1), a critical signaling pathway associated, among other processes with l-DOPA-induced dyskinesia (LID)in hemiparkinsonian animal models. Despite the growing interest for the involvement of Rhes in striatal medium spiny neurons (MSNs) dysfunction, many basic issues still need to be addressed, including specific localization and activity of Rhes in other distinct striatal neuronal subtypes.

In the present study, we characterized the expression of Rhes mRNA across species (in rodents and human samples) and we investigated the functional role of Rhes in physiology of striatal cholinergic interneurons (ChIs).

We performed double in situ hybridization analysis in rodents and human samples, and electrophysiological patch-clamp recordings coupled with calcium-imaging measurement in a Rhes knockout mouse model.

Double in situ hybridization analysis showed for the first time that Rhes transcript is selectively localized in ChIs, but not in GABAergic parvalbumin- or in neuropeptide Y-positive cell populations. Rhes is closely linked to dopamine-dependent signaling. Therefore, we recorded ChIs activity in basal condition and following dopamine receptor activation. Surprisingly, instead of an expected dopamine D2 receptor (D2R)-mediated inhibition, we observed an aberrant excitatory response in ChIs from Rhes knockout mice. Conversely, the effect of D1R agonist on ChIs was less robust in Rhes mutants than in controls. Although Rhes deletion in mutants occurs throughout the striatum, we demonstrate that the D2R response is altered specifically in ChIs, since it was recorded in

pharmacological isolation, and prevented either by intrapipette BAPTA or by GDP-β-S. Moreover, we show that blockade of Cav2.2 calcium channels prevented the abnormal D2R response.

Finally, we found that the abnormal D2R activation in ChIs was rescued by selective PI3K inhibition thus suggesting that Rhes functionally modulates PI3K/Akt signaling pathway in these neurons.

Our findings reveal that, besides its expression in MSNs, Rhes is selectively localized also in striatal ChIs and, most importantly, lack of this G-protein, significantly alters D2R modulation of striatal cholinergic excitability.

P 29/10

VGF PEPTIDES IN THE NSC-34 MOTONEURONAL CELL LINE: SELECTIVE MODULATION UPON OXIDATIVE STRESS AND POTENTIAL NEUROPROTECTIVE ROLE OF TLQP-21.

Pilleri Roberta, Brancia Carla, Noli Barbara, Cocco Cristina, Sogos Valeria, Ferri Gian Luca

Università degli studi di Cagliari

The VGF gene encodes a protein precursor (617/615 AA rat/man, respectively), which is processed in vivo to various low MW peptides with different biologic activities. These include attenuation of excytotoxic injury on spinal motor neurons from G93A-SOD-1 mice, upon overexpression of the VGF precursor, or an anti-apoptotic action of the VGF peptide TLQP-21 on cerebellar granules. Recently, the C3a and gC1q have been identified as TLQP-21 receptors.

Using the mouse motoneuronal cell line NSC-34, we studied the expression and modulation of certain VGF peptides (VGFp) and the possible neuroprotective effect.

Oxidative stress was induced with Na Arsenite (SA: 0.5mM, 60 min) while the MTT test was used to assess cell viability. Antibodies used (for either ICC or ELISA) were specific for: the N/C-terminus of the VGF precursor, NERP-1, 2 and 3, rat VGF375-420, rat PGH, NAPPE-19 and TLQP-21, and for the C3a/gC1q TLQP receptors. Both antibody receptors were also used for ICC and western blot analysis.

In NSC-34, almost all VGF peptides were selectively localized in growth cones, perinuclear region, and/or in a specific paranuclear region. Upon oxidative stress, most cells showed a rounded morphology, with loss of growth cones, while almost all VGFp were found mainly in a paranuclear region compatible with the Golgi apparatus. In ELISA, untreated cellular extracts showed the presence of the majority VGFp with a significant reduction in TLQP and NERP-1 levels versus untreated cells ($p \leq 0.31 \times 10^{-5}$ and $p \leq 0.007$, respectively) after SA treatment, while the culture medium showed a marginal increase of the same peptides. Cell viability studies showed a significant protective action of TLQP-21 on stressed neurons (SA alone: 74.7% ±0.08, SA and TLQP-21 together: 82.1% ±0.08 of untreated controls, p±0.018), with no detectable effect for NERP-1.
Results from immunocytochemistry showed the presence in NSC-34 of both receptors, C3a and gC1q, with a more marked staining for gC1q receptor, confirmed also by western blot analysis.

In conclusion, among the VGFp studied, the majority is present in NSC-34, but only TLQPp are modulated and secreted after oxidative stress, with a potential neuroprotective role. Hence, future experiments should be done to evaluate the action of TLQP-21 as protective peptide in neurodegenerative deseases.

P 30/10

A382T TARDBP GENE MUTATION ALTERS STRESS GRANULE DYNAMIC IN CULTURED FIBROBLASTS FROM ALS PATIENTS

Coni Paola[1], Brancia Carla[1], Floris Andrea[2], Bellini Ilaria[2], Orrù Sandro[2], Sogos Valeria[1]

[1]Dept. of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]Medical Genetics, Dept. Medical Sciences, University of Cagliari ~ Cagliari ~ Italy

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons. The RNA-binding protein TDP-43 (encoded by TARDP gene) is strongly linked to ALS, since it is a major constituent of pathological intracellular inclusions in this disease. Moreover, mutations in TARDP cause both sporadic and familial ALS. This protein interacts with mRNA binding proteins that are known to associate with stress granules. Stress granules are non-membranous structures, composed of non-translating messenger ribonucleoproteins, that rapidly appear in cells exposed to various types of stress and disperse after the stress is removed.

The aim of this work was to investigate the dynamic of stress granule assembly in human cultured fibroblasts from ALS patients carrying A382T TARDBP gene mutation compared with fibroblasts from healthy controls.

Cultured fibroblasts were treated with 0.5 mM sodium arsenite (SA), for 30 and 60 min, to induce stress granule formation, and immunostained for stress granule markers (TIA-1 and HuR). Cell viability after treatment was analysed by MTT assay.TARDBP silenced fibroblasts, obtained after transfection with a specific siRNA, were treated with SA as already described and double immunostained for TDP-43 and HuR.

We observed stress granules after treatment in both TARDBP A382T and wt fibroblasts, but the number of cells exhibiting stress granules was significantly lower in fibroblasts from ALS patients compared to healthy controls; moreover, the number of stress granules per cell was significantly higher in fibroblasts from healthy controls compared with those from ALS patients. When SA was removed from the culture medium, stress granules gradually dispersed in both A382T TARDBP and wt fibroblasts. MTT assay showed a significant higher cytotoxicity in fibroblasts from ALS patients compared with healthy controls; moreover we found that fibroblasts from healthy controls were able to recover after the removal of stress, while those from ALS patients were not. TDP-43 silenced fibroblasts showed a lower ability to form stress granules after SA treatment, compared with control fibroblasts, confirming the involvement of TDP-43 in stress granule dynamic.

Our data indicate that TDP-43 may modulate assembly of stress granules, and suggest that A382T TARDBP mutation may compromise the cellular stress response, contributing to neuronal vulnerability in ALS.

P 31/10

IMPAIRMENT OF SPIKE TIMING DEPENDENT PLASTICITY AT IMMATURE MOSSY-FIBER–CA3 SYNAPSES OF MICE CARRYING THE R451 MUTATION OF NL3 IS DUE TO A LOSS OF FUNCTION

Sgritta Martina[2], Bialowas Andrzej Jan [1], Wiera Grzegorz[1], Cherubini Enrico[2]

[1]Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati, (SISSA) ~ Trieste ~ Italy, [2]European Brain Research Institute (EBRI) ~ Roma ~ Italy

Autism Spectrum Disorders (ASDs) are neuro-developmental disorders, characterized by impaired social interactions, communication deficits and stereotyped behaviors. Unlike idiopathic, syndromic forms of ASDs, of genetic origin, constitute only 10 % of cases. These include rare cases with single mutations of genes encoding for synaptic proteins, such as the R451C mutation of the neuroligin (NL) 3. Neuroligins are adhesion molecules that interacting with their presynaptic partners neurexins, ensure the cross talk between the post- and pre-synaptic specializations and the maintenance of an appropriate E/I balance. Preliminary data from our laboratory have demonstrated that the R451C mutation of the Nlgn3 gene selectively affects GABAergic signaling and correlated network activity in the hippocampus and somatosensory cortex from birth (Pizzarelli and Cherubini, Frontiers in Cell Neurosci, 2013; Cellot and Cherubini, Physiol Rep, 2014).

We aimed to test the hypothesis that in mice carrying the R451C mutation of NL3 (NL3R451C knock-in mice, an animal model of Autism), at mossy fiber (MF)-CA3 synapses, that at early developmental stages are mainly GABAergic, spike timing dependent plasticity, a Hebbian form of learning, is altered.

Here, we took advantage of the whole cell configuration of the patch clamp technique to record evoked responses in control and transgenic littermates.

While in littermate controls the pairing procedure induced an increase in amplitude of MF-dependent GABAergic currents which 30 min after pairing attained 305 ± 40% of the baseline value ($p \leq 0.05$, Wilcoxon signed-rank test; $n = 7$), in NL3R451C KI mice the same protocol failed to induce LTP. Instead a persistent depression was observed (30 min after pairing the amplitude of the synaptic currents were 83 ± 18 % of the baseline value ($p > 0.05$, Wilcoxon signed-rank test; $n = 6$).Similar experiments performed on NL3 knock-out mice revealed as in KI mice a persistent reduction in amplitude of MF-mediated GABAergic currents (62.37 ± 10 % of the baseline value; $p<0.002$; $n =9$).

These results strongly suggest that the impairment of synaptic plasticity observed at immature MF-CA3 synapse in NL3R451CKI mice can be attributed to a loss of function maybe dependent on alterations of spike precision following changes in GABAergic signaling.

P 32/10

MOLECULAR MECHANISMS INVOLVED IN MITOCHONDRIAL DYNAMICS IN AN IN VITRO MODELS OF BRAIN ISCHEMIA

Ianniello Gaetano, Sisalli Maria Josè, Annunziato Lucio, Scorziello Antonella

University of Naples "Federico II" Department of Neuroscience, Reproductive and Dentistry Sciences ~ Napoli ~ Italy

Mitochondria play an important role in ATP synthesis, in the activation of apoptosis and in the regulation of calcium homeostasis. Mitochondrial dysfunction is a common feature of many pathologic conditions, such as neurodegenerative disorders. It has been demonstrated that NCX3, the only isoform of sodium/calcium exchanger found at mitochondrial level, regulates the mitochondrial Ca^{2+} homeostasis and prevents the hypoxia-induced cell death through its interaction with the protein kinase A anchoring protein 121 (AKAP121). Interestingly, the expression levels of NCX3 and AKAP121 decrease during hypoxia and mitochondrial morphology is altered. In particular, hypoxia induces the proteasomal degradation of AKAP121 through the activation of the E3-ubiquitin ligase Seven in-absentia homolog 2, Siah2.

The purpose of the present study was to understand the role played by NCX3 and AKAP121 in the regulation of the mitochondrial morphology in an in vitro model of brain ischemia.

The expression levels of Drp1 and Mfn1, two proteins involved in mitochondrial fission and fusion events, respectively, were evaluated through Western Blotting on lysates of cortical neurons obtained from WT, ncx3-/- and siah2-/- mice exposed to 3 hrs of oxygen and glucose deprivation (OGD) and OGD followed by 24 hrs of reoxygenation (OGD/Reoxy). Mitochondrial function was assessed in the above mentioned cellular models through evaluation of mitochondrial membrane potential, mitochondrial calcium levels.

The results obtained demonstrated that in basal conditions in ncx3-/- neurons Mfn1 expression was increased while Drp1 expression was reduced in comparison with ncx3+/+ neurons, however the exposure to OGD and OGD/Reoxy did not induce differences in Drp1 and Mfn1 expression between ncx3+/+ and ncx3-/- neurons, leading to hypothesize the involvement of this protein in the regulation of mitochondrial morphology during hypoxia. As matter of fact, the exposure of siah2+/+ neurons to OGD induced a reduction in NCX3 and Mfn1 expression, while Drp1 expression was increased and mitochondrial calcium content increased. Interesting, in siah2-/- neurons exposed to OGD no changes in NCX3, as well as in Drp1 and Mfn1 expression occurred. Moreover, in these conditions mitochondrial calcium concentration was lower compared to siah2+/+ neurons. The exposure to OGD/Reoxy increased Mfn1 without changes in Drp1 and NCX3 expression in both siah2+/+ and siah2-/- neurons. Once again, during reoxygenation mitochondrial calcium content in siah2+/+ and siah2-/- neurons was reduced compared to the levels detected in siah2+/+ neurons exposed to OGD.

Collectively, these findings suggest that NCX3 and AKAP121 might influence mitochondrial dynamics through the regulation of mitochondrial calcium concentrations in response to hypoxia.

P 33/10

CHARACTERIZATION OF THE NEURONAL ALTERATIONS IN THE SHANK1/SHANK3 DOUBLE KNOCK OUT MOUSE AS ANIMAL MODEL FOR SHANKOPATHIES

Mossa Adele[1], Ponzoni Luisa[2], Culotta Lorenza[1], Sala Mariaelvina[2], Sala Carlo[1], Verpelli Chiara[1]

[1]Department of Medical Biotechnology and Translational Medicine, University of Milan; CNR - Institute of Neuroscience ~ Milan ~ Italy, [2]Department of Medical Biotechnology and Translational Medicine, University of Milan ~ Milan ~ Italy

Shank family proteins (Shank1, Shank2 and Shank3) are large scaffold proteins located to the excitatory glutamatergic synapses, where they constitute an indispensable frame for the build of the postsynaptic density. Since Shank proteins play a crucial role in regulating synaptic structure and function, it is not surprising that alterations in Shank protein expression are related with abnormal brain development, resulting in different neuronal diseases termed Shankopathies. Human genetic studies show that mutations in all three SHANK genes are directly associated with neurodevelopmental disorders, such as autism spectrum disorders (ASD) and intellectual disability (ID), providing an immediate link between synaptic dysfunction and pathophysiology of these disorders.

In our project we have generated the Shank1/Shank3 double knock out (DKO) mouse as a model to further investigate the role of Shank proteins in synaptic formation, maturation, function and in the pathogenesis of ASD and ID.

To evaluate Shank1/3 DKO mice phenotype, behavioral and biochemical analysis were performed in young and adult mice. The test battery used for behavioral screening includes: social phenotype, learning and memory, emotional, as well as neurological and motor phenotype assays. The biochemical analysis were performed in different brain areas associated with ASD and ID pathogenesis.

Behavioral analysis of Shank1/3 DKO mice revealed impairments in motor performances, social interactions, learning and memory properties that can be associated to the core symptoms of ASD patients. Biochemical analysis showed altered expression of group I mGluRs in DKO mice, suggesting that allosteric modulation of these receptors may represent a potential pharmacological target for ASD and ID. Intriguingly, Shank1 knock out/Shank3 heterozygous mice, which have a similar phenotype to DKO animals, show increased repetitive behaviors and susceptibility to seizures indicating that the expression level of different Shank proteins may affect different brain functions.

Our results indicate that the Shank1/3 DKO mouse represents a good model both to better understand the role of SHANK genes in brain development and functions and to develop a rationale for new pharmacological therapies for patients carrying mutations in SHANK genes.

P 34/10

CHARACTERIZATION OF SELECTIVE MIRNAS EXPRESSION IN BLOOD AND BRAIN SAMPLES AFTER THE NEUROPROTECTIVE APPROACH OF REMOTE POSTCONDITIONING

Cepparulo Pasquale, Vinciguerra Antonio, Cuomo Ornella, Brancaccio Paola, Di Renzo Gianfranco, Annunziato Lucio, Pignataro Giuseppe

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, "Federico II" University of Naples ~ Naples ~ Italy

Ischemic stroke is a multifaceted pathology that involves gene reprogramming. Among those genes whose expression is influenced by cerebral ischemia can be included the plasmamembrane protein sodium-calcium exchanger-1 (NCX1), whose activity is tightly related to stroke outcome. We have recently identified a microRNA (miR-103-1), that can serve as stroke druggable target, able to selectively modulate NCX1 expression in the brain during stroke. Furthermore, it has been recently demonstrated that a short occlusion of an artery in a separate district of the body is able to protect the brain from a previous harmful ischemic insult: a phenomenon termed “remote postconditioning”.

To identify specific miRNAs, in blood and brain samples, able to modulate NCX expression and modulated after the neuroprotective approach of remote limb postconditioning.

Transient focal ischemia was induced in male rats by temporal occlusion of the middle cerebral artery (tMCAO); remote postconditioning was achieved by temporary occlusion of femoral artery. Blood and brain samples were withdrawn from tail vein at different time intervals from reperfusion. A specific Serum/Plasma Kit was used for microRNAs extraction from plasma. The quantification of specific isolate miRNAs was performed by real-time PCR. Brain damage was evaluated measuring the infarct volume and neurological deficit scores.

The first step has been to understand whether miR-103-1 expression was affected by stroke in plasma samples at different time intervals from reperfusion (-70min, 30min, 6h and 24h). Preliminary data show that miR-103-1 is strongly increased in plasma of rats subjected to tMCAO, suggesting that this miRNA is released from brain in the periphery.

The goal of this research plan will be (1) the identification of a miRNA cluster significantly up- or down-regulated by stroke at the brain level and released in blood serum after ischemic stroke, to have a diagnostic marker profile, and (2) the identification of microRNAs released selectively in blood serum after remote femoral postconditioning to identify miRNAs involved in neuroprotection.

P 35/10

MICROVESICLES IN NEURODEGENERATIVE DISEASES: A CROSS-TALK AMONG DIFFERENT CELL TYPES

La Salvia Sabrina[1], Sproviero Daisy[2], Colombo Federico[3], Pansarasa Orietta[2], Gagliardi Stella[2], Porretti Laura[3], Cereda Cristina[2]

[1]Department of Brain and Behavioral Sciences, University of Pavia ~ Pavia ~ Italy, [2]Laboratory of Experimental Neurobiology, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [3]Clinical Chemistry and Microbiology Laboratory, Flow Cytometry and Experimental Hepatology Service, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico ~ Milan ~ Italy

The lack of biomarkers in neurodegenerative diseases makes it impossible to determine the stage of the illness in patients, delaying therapeutic trials. Blood contains microvesicles (MVs), pro-inflammatory vesicles released by various cell types, which may serve as potential biomarkers for diagnostic and prognostic use. Microvesicles transfer mRNA, non-coding RNA (miRNA, LncRNA), or transcription factors among different cell types and this facilitates the spreading of the disease through the delivery of genetic material and pathogenic proteins. A new hypothesis about disease transmission connects several neurological diseases (AD, PD, ALS), associated with protein misfolding and aggregation, in the statement of “prion-like diseases”. It has been demonstrated that microvesicles from prion-infected neuronal cells initiate prion propagation in uninfected cells, underlying a new mechanism of disease propagation.

The aim of our study is to investigate microvesicles in plasma of ALS and AD patients, in order to discover new biomarkers of these two diseases.

Venous blood from 3 ALS, 3 AD patients and 5 healthy volunteers was centrifuged (1000 ×g for 10 minutes, 1600 ×g for 20 minutes) and the plasma was then centrifuged at 20,000 ×g for 1 hour. MVs were immunolabeled and analyzed by flow cytometry. Markers for MVs of leukocyte (CD45), endothelial (CD31), platelet (CD61), erythrocyte (CD235a) derivation and the apoptotic marker, annexin V were used.

We analyzed the percentages of annexin-V positive(+) MPs, derived from different cellular sources. First, we investigated the percentage of MVs in three healthy individuals and the data resulted consistent among them. Then, we analyzed the levels of MPs, derived from various cell types in plasma of patients with AD, ALS and healthy controls. The levels of annexin-V+ MPs and the CD235a+ annexin-V+MVs resulted enhanced, respectively of 2 and 3 fold in the plasma from AD patients compared with healthy controls. A higher concentration of annexin-V+CD61+ and a slight increase of annexin-V+ CD45+ MVs was detected in the plasma of ALS patients. Annexin-V+ CD31+ MVs were decreased in the plasma of ALS and AD patients.

Our preliminary data show that MPs could have a relevant role in the disease propagation of AD and ALS and could explain the spread of the disease from cell to cell. However, to support this idea the analysis of the number and origin of these microvesicles in plasma of more samples could be useful to monitor disease activity and therapy response in patients with AD and ALS.

P 36/10

EFFECT OF PURIFIED MURINE NGF ON ISOLATED PHOTORECEPTORS OF A RODENT DEVELOPING RETINITIS PIGMENTOSA

Rocco Maria Luisa[1], Balzamino Bijorn Omar[2], Petrocchi Passeri Pamela[1], Micera Alessandra[2], Aloe Luigi[1]

[1]Institute of Cell Biology and Neurobiology, CNR, Rome, Italy ~ ROME ~ Italy, [2]IRCCS-G.B. Bietti Foundation, Rome, Italy ~ ROME ~ Italy

A number of different studies have shown that neurotrophins, including nerve growth factor (NGF) support the survival of retinal ganglion neurons during a variety if insults. Recently, we have reported that eye NGF administration can protect also photoreceptor degeneration in a mice and rat with inherited retinitis pigmentosa.

However, the evidence that NGF acts directly on photoreceptors and that other retinal cells mediate the NGF effect could not be excluded. In the present study we have isolated retinal cells from rats with inherited retinitis pigmentosa (RP) during the post-natal stage of photoreceptor degeneration.

A total of 96 RCS animals at postnatal day 10 (p10) were housed at the CNR animal facility and handled according to the experimental procedure approved by the Ethical Commission on animal experimentation of the National Research Council (CNR, Rome). All experiments were conducted in accordance with the guidelines for the use of animals stated by the Association for Research in Vision and Ophthalmic Research. All efforts were made to reduce the total number of animals and to minimize animal suffering. At the time of sampling, animals were sacrificed by means of an overdose of anaesthetic (ketamine-xylazine and tiletamine-zolazepam; Fort Dodge Veterinaria, S.A., Vall de Bianya-Girona, Spain).

In presence of NGF, these cells are characterized by enhanced expression of NGF-receptors and rhodopsin, the specific marker of photoreceptor and better cell survival, as well as neuritis outgrowth.

Together these observations support the hypothesis that NGF acts directly on photoreceptors survival and prevents photoreceptor degeneration as previously suggested by in vivo studies.

P 37/10

PHARMACOLOGICAL RESCUE OF KCNQ2 CHANNELS CARRYING EARLY-ONSET EPILETIC ENCEPHALOPATHY MUTATIONS

De Maria Michela[1], Soldovieri Maria Virginia[1], Ambrosino Paolo[1], Mosca Ilaria[1], Miceli Francesco[2], Striano Pasquale[3], Weckhuysen Sarah[4], Cooper Edward C[5], Taglialatela Maurizio[1]

[1]University of Molise, Dept. of Medicine and Health Science ~ Campobasso ~ Italy, [2]University of Naples ~ Department of Neuroscience ~ Naples ~ Italy, [3]University of Genoa, Dept. of Neuroscience ~ Institute "G.Gaslini", Genoa ~ Italy, [4]Neurogenetics Group ~ Department of Molecular Genetics, Antwerp ~ Belgium, [5]Baylor College of Medicine ~ Dept. of Neuroscience and Molecular and Human Genetics, Houston ~ United States

Kv7.2/3 channels underlie the M-current (IKM), a potassium-selective neuronal current characterized by low activation threshold, slow activation and deactivation kinetics, and absence of inactivation. Mutations in Kv7.2/3 are responsible for genetically-determined epileptogenic diseases in neonates, showing a wide phenotypic heterogeneity, ranging from Benign Familial Neonatal Seizures (BFNS) to severe Early-Onset Epileptic Encephalopathy (EOEE). The molecular basis for such phenotypic heterogeneity is unknown, but the mutation-induced functional changes in the ionic currents seem to play a major role.

In the present work, the biochemical and functional consequences prompted by Kv7.2 mutations (A265T, R325G, or S195P) found in EOEE-affected patients have been investigated; in parallel, the ability of Kv7 modulators to counteract mutation-induced alterations has also been studied.

Mutations were engineered by site-directed mutagenesis in a plasmid for mammalian expression containing the cDNA for the human isoform of Kv7.2 subunits and mutant plasmids were transiently transfected in Chinese Hamster Ovary (CHO) cells. After 24 h, these cells were used either for western-blot experiments on total lysates or plasma-membrane fractions, or in electrophysiological experiments by the whole-cell configuration of the patch-clamp technique.

Patch-clamp recordings revealed that homomeric KCNQ2 A265T or R325G mutant channels were non-functional. When mutant subunits were expressed in heteromeric configuration with WT KCNQ2 and KCNQ3 subunits to reproduce the genetic balance of affected individuals, a significant reduction in maximal current density was measured, suggesting a loss-of-function as a pathogenetic mechanism. Biotinylation assays revealed that mutation-induced currents reduction was not due to changes in plasma membrane levels of mutant subunits. Exposure to the Kv7 opener retigabine (10 μM) significantly increased KCNQ2/KCNQ2 A265T/KCNQ3 or KCNQ2/KCNQ2 R325G/KCNQ3 current density, rescuing defective channels to wild-type levels. By contrast, KCNQ2 S195P mutant subunits, both when expressed in homomeric or heteromeric configuration with KCNQ2/3 subunits, showed a significant increase in the maximal current density and a robust leftward shift in the voltage-dependence of activation, revealing that a gain-of-function mechanism is instead associated to this mutation, as it occurs with other mutations affecting the

proximal S4 region. Exposure to the potent and selective KCNQ2 blocker ML252 (100 nM) similarly reduced by about 50% the currents carried by WT or KCNQ2 S195P homomeric channels.

Altogether, these results suggest that EOEE-associated mutations can lead to divergent biophysical consequences on KCNQ2 channels, ranging from gain- to loss-of function effects. These mutation-specific effects should guide patient-tailored pharmacological treatments.

P 38/10

GENETIC VARIATION IN RASD2 MODULATES PSYCHOTOMIMETIC DRUG EFFECTS IN MICE AND SCHIZOPHRENIA-RELATED PHENOTYPES IN HUMANS

Napolitano Francesco[1], Vitucci Daniela[4], Di Giorgio Annabella[2], Errico Francesco[1], Attrotto Maria Teresa[3], Gelao Barbara[3], Fazio Leonardo[3], Di Maio Anna[4], Marsili Valentina[4], Taurisano Paolo[3], Blasi Giuseppe[3], Pelosi Barbara[5], Pasqualetti Massimo[5], Bertolino Alessandro[3], Usiello Alessandro[6]

[1]University of Naples "Federico II",Department of Molecular Medicine and Medical Biotechnology ~ Napoli ~ Italy, [2]IRCSS Casa Sollievo della Sofferenza, San Giovanni Rotondo ~ Foggia ~ Italy, [3]Group of Psychiatric Neuroscience, University of Bari "Aldo Moro" ~ Bari ~ Italy, [4]Lab of Behavioural Neuroscience, CEINGE Biotecnologie Avanzate ~ Napoli ~ Italy, [5]Dept of Biology, University of Pisa ~ Pisa ~ Italy, [6]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples (SUN) ~ Caserta ~ Italy

The GTP-binding protein Rasd2 is highly expressed in the striatum and involved in the regulation of dopamine signaling within striatal GABAergic projection neurons and cholinergic interneurons. Moreover, Rasd2 also modulates different key neurodevelopmental pathways, including AKT1 and mTOR, implicated in psychiatric disorders. Noteworthy, the human ortholog RASD2 is located on the long arm of chromosome 22(q12.3), a genomic region associated to several susceptibility loci for psychosis.

In the present study, first we investigated if Rasd2 is also expressed in human prefrontal cortex (PFC), and then if RASD2 genetic variation modulates a series of prefrontal and striatal phenotypes in rodents and humans.

Coronal sections of rodent and human post-mortem brains were obtained in the region of putamen and PFC and analyzed through in situ hybrydization. Prepulse inhibition (PPI) of the acoustic startle response was used to evaluate sensorimotor gating in Rasd2 KO male mice and their respective WT controls, under both basal condition and following amphetamine or phencyclidine administration. 216 post-mortem brains of non-psychiatric individuals were analyzed (http://braincloud.jhmi.edu). Association of RASD2 SNPs (N=66) with RASD2 mRNA expression was explored (Bonferroni correction). Rs6518956 (G/A) (intronic SNP) was selected for further in vivo analysis with imaging tools. 150 healthy subjects underwent 3T sMRI to test the association of rs6518956 with prefrontal grey matter (GM) volume as assessed with voxel based morphometry (VBM). 160 healthy subjects underwent 3T BOLD fMRI to test the association of rs6518956 with prefrontal activity during Working Memory (WM) performance (N-Back task).

We first confirmed a consistent Rasd2 mRNA expression in the mouse striatum, and we found that human RASD2 mRNA occurs in putamen and in cortical regions throughout layers II to VI, with a striking specie-specific pattern in PFC, showing a greater expression within layer V pyramidal neurons. In addition, naïve Rhes KO mice displayed sensorimotor gating deficits, exacerbated by amphetamine or PCP administration. Finally, we showed that a non-coding variation in the RASD2 gene (rs6518956), which affects RASD2 mRNA levels in human prefrontal post-mortem tissue,

also predicts in vivo phenotypes of prefrontal GM volume, as well as prefrontal and striatal activity during WM processing in healthy subjects.

Collectively, our results suggest that Rasd2 modulates prefrontal and striatal phenotypes in rodents and humans. Relevance of these findings for the pathophysiology of schizophrenia should be further elucidated.

P 39/10

EFFECT OF RESVERATROL ON PLASMATIC MOLECULAR INDICATORS OF BRAIN TISSUE RESPONSE TO THE HYPOPERFUSION/REPERFUSION CHALLENGE

Poddighe Laura[1], Serra Maria Pina[1], Boi Marianna[1], Picci Cristina[1], Carta Gianfranca[2], Murru Elisabetta[2], Lisai Sara[2], Sirigu Annarita[2], Collu Maria[3], Banni Sebastiano[2], Quartu Marina[1]

[1]Dipartimento di Scienze Biomediche, sez. di Citomorfologia, Università di Cagliari ~ Cagliari ~ Italy, [2]Dipartimento di Scienze Biomediche, sez. di Fisiologia, Università di Cagliari ~ Monserrato (CA) ~ Italy, [3]Dipartimento di Scienze Biomediche, sez. di Neuroscienze, Università di Cagliari ~ Monserrato (CA) ~ Italy

Endocannabinoids (eCBs) and congeners show a neuroprotective role in several experimental models of brain injury and several lines of evidence indicate that changes in eCB levels in peripheral blood cells may reflect the severity of neurological insult. We have previously shown that the preventive administration of dietary natural compounds may increase the plasmatic levels of palmytoylethanolamide (PEA) and oleoylethanolamide (OEA) following the transient bilateral common carotid artery occlusion (BCCAO)-induced brain tissue challenge. Resveratrol (RVT), (3,4',5-trihidroxystilbene) is a strong natural antioxidant of polyphenolic structure found in grapes and red wine, with many physiological effects, including the prevention of lipid peroxidation in human LDL, inhibition of arachidonic acid metabolism, and platelet activity. RVT has been further shown to protect cerebral tissue and cardiac muscle from tissue damage caused by oxidative stress triggered by reperfusion and has been proposed as a potential neuroprotective agent in treating acute states in focal cerebral ischemia injury.

To evaluate whether exogenous administration of RVT prior to induction of BCCAO followed by reperfusion (BCCAO/R) influences the molecular changes occurring in cerebral cortex and plasma, with particular focus on the eCB system.

Cerebral transient hypoperfusion was produced by a 30 min BCCAO followed by 60 min reperfusion. Animals were starved for 12 hours before surgery and 6 hours prior to ischemia RVT (40 mg/kg/0.45 ml of sunflower oil as vehicle) was administered via gavage. Biological samples of plasma, cerebrospinal fluid (CSF), and brain tissue were examined by HPLC, gel zymography, western blot and immunohistochemistry.

Data obtained indicate that RVT appears to influence the outcome of BCCAO/R cerebral injury by modulating changes in the concentrations of eCBs and eCB congeners, lipid hydroperoxides, markers of oxidative stress, and changes in the expression of CB1 and CB2 receptors, peroxisome proliferator-activated receptor-(PPAR) alpha, ciclooxygenase-2 (COX-2) protein levels and enzymatic activity of matrix-metalloproteinase-9 (MMP-9). Interestingly, changes in brain of some of these parameters, like lipid hydroperoxides, were also found in plasma.

Data obtained suggest that exogenous administration of RVT may modulate the brain tissue compensatory or repair mechanisms triggered by the hypoperfusion/reperfusion and support the possible use of this molecule as treatment to prevent the BCCAO/R-induced brain insult. Changes in plasma mirrored those found in cerebral tissue, opening to the possibility to test whether RSV exerts its positive activities in humans.

P 40/10

RESVERATROL VIA SIRTUIN-1 DOWNREGULATES RE1-SILENCING TRANSCRIPTION FACTOR (REST) EXPRESSION PREVENTING PCB-95- INDUCED NEURONAL CELL DEATH

Laudati Giusy[1], Guida Natascia[2], Anzilotti Serenella[2], Montuori Paolo[3], Di Renzo Gianfranco[1], Canzoniero Lorella Maria Teresa[4], Formisano Luigi[4]

[1]Division of Pharmacology, Department of Neuroscience, Reproductive and and Dentistry Sciences, School of Medicine, "Federico II" University of Naples, Via Pansini, 5, 80131 ~ Naples ~ Italy, [2]IRCSS SDN, 80131 ~ Naples ~ Italy, [3]Department of Public Health, 'Federico II' University of Naples ~ Naples ~ Italy, [4]Division of Pharmacology, Department of Science and Technology, University of Sannio, via Port'Arsa 11, 82100 ~ Benevento ~ Italy

Resveratrol (RSV), a polyphenol present in plants, exerts a neuroprotective function in several neurological conditions; it is an activator of class III histone deacetylase sirtuin1 (SIRT1), a crucial regulator in the pathophysiology of several neurodegenerative diseases such as stroke, Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease. By contrast, the RE1-silencing transcription factor (REST) is involved in the neurotoxic effects following exposure to polychlorinated biphenyl (PCB) mixture A1254.

We investigated the effects of RSV (SIRT1 activator) and EX-527 (SIRT1 inhibitor) on REST mRNA and protein expression. Furthermore, we identified the molecular mechanism by which RSV-regulated REST expression. In addition, we studied the relationship between the non-dioxin-like (NDL) PCB-95 and REST through SIRT1 to regulate neuronal death in rat cortical neurons.

Human neuroblastoma SH-SY5Y cells and rat cortical neurons (DIV7) were treated with RSV, EX-527 and PCB-95 at different concentrations. ChIP assays were used to study the interaction between transcription factors and REST human promoter sequence. q-RT-PCR and Western Blot were utilized to evaluate the mRNA and protein expression. Gene knock-down was performed by siRNAs transfection. Lactate dehydrogenase assay was used to assess cell death.

RSV significantly decreased REST gene and protein levels in a dose- and time-dependent manner. Interestingly, overexpression of SIRT1 reduced REST expression, whereas EX-527, an inhibitor of SIRT1, increased REST expression and blocked RSV-induced REST downregulation. These results suggest that RSV downregulates REST through SIRT1. In addition, RSV enhanced activator protein 1 (AP-1) transcription factor c-Jun expression and its binding to the REST promoter gene. Indeed, c-Jun knock-down reverted RSV- induced REST downregulation. Intriguingly, in SH-SY5Y cells and rat cortical neurons the NDL PCB-95 induced necrotic cell death in a concentration-dependent manner by increasing REST mRNA and protein expression. In addition, SIRT1 knock-down blocked RSV-induced neuroprotection in rat cortical neurons treated with PCB-95.

Collectively, these results indicate that RSV via SIRT1 activates c-Jun, thereby reducing REST expression in SH-SY5Y cells under physiological conditions and blocks PCB-95-induced neuronal cell death by activating the same SIRT1/c-Jun/REST pathway.

P 41/10

TRANSPLANTED ADULT STEM/NEUROPROGENITOR CELLS (ANPCS) IN RODENT MODELS OF PARKINSON'S DISEASE: CHARACTERIZATION, PROPERTIES AND IN VIVO EVIDENCE OF NIGROSTRIATAL DOPAMINERGIC NEURORESTORATION

L'Episcopo Francesca[1], Tirolo Cataldo[2], Testa Nunzio[2], Serapide Maria Francesca[3], Peruzzotti Jametti Luca[4], Pluchino Stefano[4], Marchetti Bianca[1]

[1]OASI Inst. for Res and Care (IRCCS), Neuropharmacol. Section; Dept. of Biomed. and Biotechnol Sci., Pharmacology Sections, Univ. of Catania Medical School. ~ Catania ~ Italy, [2]OASI Inst. for ResCare (IRCCS), Neuropharmacol. Section ~ Troina (EN) ~ Italy, [3]Dept. of Biomed. and Biotechnol Sci, Physiology Sections, Univ. of Catania Medical School ~ Catania ~ Italy, [4]Dept. of Clinical Neurosciences, Cambridge Centre for Brain Repair and Cambridge Stem Cell Initiative, Univ of Cambridge, ED Adrian Building ~ Cambridge ~ United Kingdom

Neural stem/progenitor cells (NPCs) are powerful research tools for the design and the discovery of new approaches to cell therapy in neurodegenerative diseases, such as Parkinson's disease (PD). To this aim, the molecular mechanisms that govern neurogenesis and differentiation of midbrain dopaminergic (mDA) neurons have attracted intense investigations for the identification of intrinsic dopaminergic (DA) neuron determinants. Specifically, Wingless-type MMTV integration site (Wnt) pathway is a chief regulator of DA neurogenesis in the developing midbrain and endogenous Wnt signaling activation contributes to adult mDA neuron maintenance. In PD, Wnt pathway is dysfunctional and mDA neurons progressively degenerate in the substantia nigra pars compacta (SNpc), leading to substantial decreases in striatal dopamine levels. New evidence indicates that the adult midbrain aqueduct periventricular regions (Aq-PVRs) harbor neural stem/progenitor cells (mNPCs) with DA potential in vitro, but restrictive mechanisms in vivo limit DA regenerative capacity.We recently provided evidence that aging is one most critical factor limiting mNPCs neurogenic potential both in vivo and in vitro, via a dysregulation of Wnt/β-catenin signaling and a loss of astrocyte-derived Wnts.

Here, we have determined wether transplantation of these adult mNPCs in 6-OHDA hemi-lesioned aged mouse model can relieve the PD features.

To this end, adult mNPCs expressing proliferation (bromodeoxyuridine, BrdU), precursor (nestin, Musashi1), proneural (oligodendrocyte transcription factor 2, Olig2; neurogenin2, Ngn2), and high levels of the midbrain marker engrailed 2 (En2) in vitro, were propagated and used for transplantation at passage 8-10 without or after activation protocols, at different time-intervals after 6-OHDA unilateral injection into the left striatum of 11-15 month old mice. The mNPCs were transplanted in the ipsilateral SNpc.

Spatio-temporal histhopathological, immunohistochemical and functional analyses from 8-45 d post-transplantation indicated the potential of the grafted mNPCs to induce a significant degree of nigrostriatal DA restoration. All this provides proof of concept for developing tools for neurorestorative approaches in PD, for cell replacement research and drug testing.

P 42/10

SHEDDING VESICLES RELEASE FROM A HUMAN MICORGLIAL CELL LINE: A TOOL FOR STUDYING MICROVESICLES BIOLOGY

Colombo Federico, Casella Giacomo, Finardi Annamaria, Furlan Roberto

San Raffaele Scientific Institute ~ Milano ~ Italy

Cell-to-cell communication, a crucial aspect of tissues physiology and homestasis, can be achieved by different ways; among them the release of microvesicles has recently attracted the attention of the investigators in the field after decades of neglection as considered mere cell artifacts. Two different types of microvesicles have been purified and characterized: shedding vesicles or ectosomes (SVs) and exosomes. The former are 150-1000 nm-sized particles released by the budding and fission of specific plasma membrane domains (liquid-ordered phase or lipid rafts) after stimuli such as ATP or intracellular calcium rises; the latter are nanovesicles (20-100 nm) derived from the limiting membrane of multivesicular bodies (MVBs) and released in the extracellular space after its exocytosis. Their roles are well established either in phisiological or pathological conditions mainly of the hematopoietic (where they were first described) and the nervous system, where oligodendrocytes-derived particles are important in the metabolic support of axons, and microglia-derived ones as regulators of neuronal membrane excitability.

In neuroinflammatory disorders, such as multiple schlerosis, the levels of myeloid-derived SVs increase dramatically in cerebrospinal fluid and their number well correlates with the disease severity. For better understanding the basic principles governing their release under normal or pathological conditions we decided to use as an in vitro model the human microglia cell line CHME5: therefore we verified the ability of these cells to release microvesicles in a regulated manner.

SVs were purified by centrifuging the conditioned cell media at 10,000 g (30 min) after a 300 g step for getting rid of detached cells and debris. The vesicles identity was confirmed by TEM observations of negative stained-10,000g pellets. The amount of SVs release was measured semi-quantitatively by western blot analysis of the lipid raft-enriched protein flotillin-1. The M1/M2 polarization state of CHME-5 cells was measured by quantyifing specific markers by real time PCR.

CHME-5 cells are able to acquire the M1/M2 phenotype (according to the current paradigm) after treatment with pro- or anti- inflammatory cytokines; moreover the same stimuli are able to trigger the relase of shedding vesicles similarly as that induced by ATP treatment.

Here we described a detailed characterization of the ability of a microglia cell line of human origin (CHME-5) to relase SVs upon ATP and afer cytokines treatment, which are stimuli mimicking the inflammatory microenvironment. The model presented seems to be useful as an in vitro platform for studying the myeloid-mediated neuroinflammatory diseases.

P 43/10

NITRATED P53 AS AN EARLY BIOMARKER OF UNBALANCE REDOX STATUS IN ALZHEIMER'S DISEASE (AD)

Maziano Mariagrazia, Abate Giulia, Uberti Daniela, Memo Maurizio

Department of Biomedical Sciences and Biotechnology, University of Brescia ~ Brescia ~ Italy

An early Alzheimer's disease (AD) diagnosis is still missing because of the difficulty to establish a standardized method in periphery. On this basis, the measurement of oxidative stress and p53 levels was made in immortalized B-lymphocytes AD patients to define a specific marker with prognostic value.

The aim of this study was to mesure oxidative stress in immortalized lymphocytes derived from AD patients to define a specific blood signature of the disease.

The levels of oxidative markers were measured (4-HNE, 3-NT and protein carbonyl) through western blot. The antioxidant activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRD) were measured by enzymatic assays. To study p53 conformations, immunoprecipitation experiments with PAb1620 (for p53 wild-type) and PAb240 (for p53 unfolded) antibodies, were performed. The effect of peroxynitrite compound (SIN-1) on p53 conformation was evaluated with FACS analysis.

We observed increased levels of HNE and 3-NT only in familiar AD, compared with controls. A reduced SOD and GRD activity was evident in familiar and sporadic AD. Furthermore a significant amount of p53 was found conformationally altered in both pathological groups in comparison with controls, demonstrated by the high reactivity to PAb240 antibody. Immunoprecipitation experiments followed by the immunoblotting with anti 3-NT antibody, showed in both groups an increase of nitrated tyrosine residues in samples immunoprecipitated with PAb240. Interestingly, the nitration of tyrosine residues of p53 could be responsible of the p53 conformational change towards an unfolded phenotype, as demonstrated by the SIN-1 experiment. A correlation between unfolded p53 and SOD activity was also found.

The oxidative stress could alter p53 conformation. Nitrated-p53 might be considered as a potential early biomarker for AD.

P 44/10

INVOLVEMENT OF CENTRAL HISTAMINERGIC SYSTEM IN THE PPAR-ALPHA LIGANDS INDUCED ANALGESIC AND ANTIDEPRESSANT-LIKE EFFECTS IN MICE

Costa Alessia, Blandina Patrizio, Ghelardini Carla, Passani Maria Beatrice, Provensi Gustavo

University of Florence ~ Florence ~ Italy

Several reports demonstrated that the treatment with endogenous agonists of the nuclear Peroxisome Proliferator-Activated Receptor alpha (PPARα), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) induced anti-inflammatory, anti-hyperalgesic and antidepressant-like effects in rodents. We recently demonstrate that neuronal histamine contributes to OEA-induced hypophagic effect and recent evidence indicates that brain histamine is involved in the pathophysiology of depression and pain transmission

In the present study we investigated if the analgesic and antidepressant-like effects of PPARα-ligands requires the integrity of the central histaminergic system in mice.

The effect of OEA and PEA treatment was evaluated in mice unable to synthesize histamine (histidine decarboxylase knock-out, HDC-KO) and in wild type littermates (WT) by using classical models predictive of antidepressant-like and antinociceptive activities: the tail suspension test (TST) and the acetic acid-induced abdominal writhing test, respectively. In the TST mice were treated with vehicle, OEA (5 or 10 mg/kg, i.p.), PEA (2.5, 5 or 10 mg/kg, i.p.) or imipramine (10 mg/kg, i.p.) using two different regimens: sub-chronic (24, 5 and 1 hour before test) and chronic (once daily for 7 days) before challenging mice in the TST; the immobility time, during 6 minutes of test, was measured. In the acetic acid-induced abdominal writhing test, WT and HDC-KO mice received a single acetic acid (0.8%, i.p.) injection 30 min after OEA (5 or 10 mg/kg, i.p.) or vehicle; the number of writhings was evaluated for 10 minutes after acetic acid injection.

The results of TST indicate that treatment with OEA at either dose and PEA at highest dose induced a dose-dependent reduction of immobility time when compared with vehicle-treated animals with both sub-chronic and chronic regimen. These effects were not observed in mice lacking neuronal histamine, since no differences were observed among groups. Writhings frequency was reduced in OEA-treated WT mice as compared with control, but the effect did not reach statistical significance.

Taken together, our data suggest that the histaminergic system contributes to PPARα-induced analgesic and antidepressant-like effects, and indicate that PPARα may be an attractive target for the development of innovative antidepressant and analgesic drugs.

P 45/10

CONFORMATIONALLY ALTERED P53 AFFECTS NEURON BIOLOGICAL FUNCTIONALITY

Abate Giulia, Marziano Mariagrazia, Uberti Daniela, Memo Maurizio

Department of Biomedical Sciences and Biotechnology, University of Brescia ~ Brescia ~ Italy

Alzheimer's disease (AD) is the most frequent form of neurodegenerative disease associated with dementia in the elderly.The role of p53 in neurodegenerative diseases is essentially associated with neuronal death. Recently an alternative point of view is emerging, as altered p53 conformation and impaired protein function have been found in fibroblasts and blood cells derived from AD patients.

In this study we investigated a possible contribution of p53 unfolded protein in neurodegeneration, as occurring in AD.

An in-vitro model of a stable transfected SH-SY5Y clone overexpressing APP751wt was used to study oxidative stress markers, such as HNE Michael-adducts and 3-Nitro-Tyrosine, as well as unfolded p53. p53 conformation was evaluated using two specific monoclonal antibodies: PAb1620 (that recognizes p53wt) and PAb240 (directed towards unfolded p53). Furthermore, growth-associated protein 43 (GAP43) was examined at mRNA and protein levels.

We found that SY5Y-APP clone expressed an increased amyloidogenic processing with enhanced expression of C-terminal fragments C99 and C83 and β-amyloid peptide. High oxidative markers and unfolded p53 conformation, due essentially to nitration of its tyrosine residues, were also observed in SY5Y-APP cells. In addition, this clone was less sensitive to acute oxidative insult because of p53 impairment. SY5Y-APP cells expressed reduced GAP-43 mRNA and protein levels in comparison with control, affecting cell differentiation and morphology. Both H_2O_2-sensitivity and GAP-43 expression were restored by modulating p53 conformation towards a wild-type phenotype. In particular, zinc supplementation reverted p53 wild-type tertiary structure and increased cells sensitivity to acute cytotoxic injury and GAP-43 levels in SY5Y-APP clone.

We propose p53 oxidation/nitration as one of the early molecular events in the establishment of neuronal dysfunction, leading to cognitive impairment and AD pathology. In particular, elevated oxidative environment may affect p53 conformation towards an unfolded structure. In this unfolded state, p53 is not able to exert its proapototic activity and physiological role in axonal outgrowth.

P 46/10

THE PROTECTION INDUCED BY L-BMAA-INDUCED PRECONDITIONING IN AMYOTROPHIC LATERAL SCLEROSIS MICE BEARING G93A MUTATION IS MEDIATED BY MODULATION OF PROTEINS CONTROLLING IONIC HOMEOSTASIS

Simeone Giuseppe[1], Anzilotti Serenella[2], Vinciguerra Antonio[1], Cuomo Ornella[1], Brancaccio Paola[1], Cepparulo Pasquale[1], Cerullo Pierpaolo[1], Annunziato Lucio[1], Pignataro Giuseppe[1]

[1]Division of Pharmacology, Department of Neuroscience, Reproductive Sciences and Dentistry. School of Medicine, University Federico II of Naples ~ Naples ~ Italy, [2]SDN IRCCS ~ Naples ~ Italy

Preconditioning (PC) is a phenomenon wherein a mild insult induces a cellular and tissue resistance to a later, severe injury. To date, although PC has been extensively studied in several neurological disorders, no studies have been performed in Amyotrophic Lateral Sclerosis (ALS). Here we hypothesize that, in analogy to other diseases, PC elicits gene expression changes leading to a state refractory to ALS. Identification of these changes would provide insight into endogenous mechanism of protection.

According to our hypothesis the aims are: (1) to characterize the first preconditioning mouse model of ALS based on subthreshold treatment with the toxin L-BMAA, whose chronic use triggers an ALS state and (2) to demonstrate that the plasmamembrane exchanger Na^+/Ca^{2+}, NCX, represents a target for setting on new strategies in ALS intervention.

The experiments were carried out on 4 groups of mice: 1) wild type; 2) mice bearing the G93A mutation; 3) mice knock out for NCX3; 4) G93A and NCX3 -/- mice. Each group of mice were randomly treated with L-BMAA or vehicle. We examined the preconditioning effect on disease onset and duration, motor functions, behavioral tests, and on motoneurons in terms of functional declines and severity of histological damage by Western Blot Analysis and confocal microscopy.

We observed 1) an increase of NCX3 levels in the brain stem and in the cervical spinal cord; 2) a peculiar NCX3 localization in several brain stem nuclei motoneurons (nucleus facialis, nucleus ambiguus, hypoglossal nucleus, trigeminal nucleus and cervical spinal cord) using confocal microscopy; 3) an increase of mice survival and a better behavioral motor task performance in G93A preconditioned mice.

These studies allowed us to setting on the first model of preconditioning in ALS and to candidate NCX3 as a new target able to prevent degeneration through the preconditioning response. Moreover, the fundamental mechanism responsible for preconditioning-induced tolerance will help in designing novel pharmacological approaches for protection.

P 47/10

A ROLE FOR ENVIRONMENTAL CHEMICALS IN AUTISM ETIOLOGY: GESTATIONAL EXPOSURE TO LOW DOSES OF THE ORGANOPHOSPHATE INSECTICIDE CHLORPYRIFOS ENHANCES OXIDATIVE STRESS IN A MOUSE MODEL OF AUTISM

De Felice Alessia[1], Minghetti Luisa[2], Greco Anita[2], Calamandrei Gemma[1]

[1]Section of Neurotoxicology and Neuroendocrinology, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità ~ Viale Regina Elena 299, I-00161, Rome, Italy ~ Italy, [2]Section of Experimental Neurology, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità ~ Viale Regina Elena 299, I-00161, Rome, Italy ~ Italy

Much evidence suggests that dysregulated immune responses, associated to enhanced oxidative stress, abnormal mitochondrial metabolism and impairments in lipid metabolism, may be implicated in the etiopathogenesis of autism spectrum disorders (ASD) and other neurodevelopmental disorders. Isoprostanes are a class of prostaglandin-like molecules that are produced by peroxidation of arachidonic acid induced by free radicals; their excessive production is generally considered as an index of oxidative stress. Prostaglandins (PGEs) are among the most important lipid signaling messengers of the brain, as they control many neural functions whose alteration may affect the nervous system.

Mice of the BTBR T+tf/J (BTBR) are a well-studied model of idiopathic autism. They display several behavioural traits relevant to ASD, paralleled by immunological alterations and enhanced oxidative stress in either peripheral and brain compartments. We hypothesize that BTBR mice might be more susceptible to oxidative stress promoted by environmental toxicants such as the organophosphate pesticides (OP).

The aim of this study was to assess whether gestational exposure to the widely diffused OP insecticide, chlorpyrifos (CPF) could have an impact on the altered oxidative stress response characteristic of the BTBR strain.

To this aim, we administered pregnant mice of the BTBR inbred strain with vehicle or CPF at the sub-toxic dose of 6 mg/kg/bw by oral gavage from gestational day (GD) 14 to 17. F2-isoprostanes and PGE2 were measured in cerebellum at postnatal day (pnd) 21 and in the brain at pnd 1, 21 and 70 in the offspring of both sexes.

We found that BTBR mice present higher baseline levels of F2-isoprostanes at birth in comparison to the B6 strain, suggestive of enhanced oxidative stress processes. Gestational treatment with CPF further amplifies this characteristic profile at birth until weaning, while it promotes a significant increase of PGE2 levels at the weaning age and adulthood. Furthermore, we found a significant sex effect at weaning only in CPF-treated mice, with males that display higher levels of 15-F2t-IsoP than females.

These findings indicate that CPF exposure alters the neuroinflammatory profile of the BTBR strain, thus supporting the hypothesis that enhanced oxidative stress might be the link between early exposure to environmental neurotoxicants and brain disorders.

P 48/10

CHF5074 (CSP-1103) INDUCES MICROGLIA ALTERNATIVE ACTIVATION IN PRIMARY GLIAL CULTURES EXPOSED TO BETA-AMYLOID AND IN PLAQUE-FREE TG2576 MICE

Porrini Vanessa[2], Lanzillotta Annamaria[1], Branca Caterina[1], Benarese Marina[1], Parrella Edoardo[1], Lorenzini Luca[3], Calzà Laura[4], Flaibani Raffaella[5], Spano Pier Franco[2], Imbimbo Bruno Pietro[6], Pizzi Marina[2]

[1]Department of Molecular and Translational Medicine, University of Brescia and National Institute of Neuroscience ~ Brescia ~ Italy, [2]Department of Molecular and Translational Medicine, University of Brescia and National Institute of Neuroscience; IRCCS San Camillo Hospital ~ Brescia; Venezia ~ Italy, [3]Health Science and Technologies, Interdepartmental Center for Industrial Research (HST-ICIR) ~ Bologna ~ Italy, [4]Health Science and Technologies, Interdepartmental Center for Industrial Research (HST-ICIR); Department of Pharmacy and Biotechnology; IRET Foundation ~ Bologna ~ Italy, [5]IRCCS San Camillo Hospital ~ Venezia ~ Italy, [6]Research & Development, Chiesi Farmaceutici ~ Parma ~ Italy

Activation of microglia associated with neuroinflammation and loss of phagocytic activity is considered to play a prominent role in the pathogenesis of Alzheimer's disease (AD). CHF5074 (CSP-1103) has been shown to reduce brain inflammation in patients with mild cognitive impairment. CHF5074 was also found to improve recognition memory and hippocampal LTP when administered to plaque-free Tg2576 mice (5-month-old) for 4 weeks.

We studied the effect of CHF5074 on the expression profile of proinflammatory (M1) and anti-inflammatory/phagocytic (M2) microglia in astrocyte-microglia cultures exposed to beta-amyloid 1-42 (Aβ) and in brain of 5-month-old Tg2576 mice.

Mouse astrocyte-microglia primary cultures were exposed to 10 µM Aβ with or without 3 µM CHF5074, for 2 or 8 days. Five-month-old Tg2576 mice were treated with CHF5074 (375ppm) or vehicle for 4 weeks.

In astrocyte-microglia cultures exposed to 10µM Aβ, CHF5074 totally suppressed the Aβ -induced pro-inflammatory expression of IL-1β, TNFα and iNOS mRNAs. Furthermore, CHF5074 significantly increased mRNA levels of the M2 anti-inflammatory Mannose Receptor type C1 (MRC1/CD206) and Triggering Receptor Expressed on Myeloid cells 2 (TREM2). The effect of CHF5074 was not reproduced by ibuprofen or R-flurbiprofen, as both compounds did not modify the anti-inflammatory/phagocytic transcription. In the hippocampus of 5-month-old Tg2576 mice we detected no increase of pro-inflammatory gene transcription, when compared to wild-type littermate, but a significant reduction of M2-related genes MRC1/CD206, TREM2 and the chitinase-3 like 3 (Ym1) expression. CHF5074 treatment did not modify M1 transcription but significantly increased expression of MRC1 and Ym1. CHF5074 effects appeared to be hippocampus-specific, as the M2 transcripts were only slightly modified in the cerebral cortex.

CHF5074 specifically drives the expression of microglia M2 markers either in young Tg2576 hippocampus or in primary astrocyte-microglia cultures, suggesting its potential therapeutic efficacy as microglial modulator in the early phase of AD.

P 49/10

NEUTRALIZATION OF THE APOPTOTIC EFFECTS OF THE NEUROTOXIC CYTOKINE TRAIL BY A NANOPARTICLE-CONJUGATED SPECIFIC MONOCLONAL ANTIBODY

Ronsisvalle Nicole[1], Di Benedetto Giulia[1], Musumeci Teresa[2], Puglisi Giovanni[2], Bernardini Renato[1], Cantarella Giuseppina[1]

[1]Department of Biomedical and Biotechnological Sciences University of Catania ~ Catania ~ Italy, [2]Department of Drug Sciences, University of Catania ~ Catania ~ Italy

Alzheimer's disease (AD) is the most common form of dementia worldwide, with scarce therapeutic options. Although many efforts have been done to neutralize its landmark neurotoxic molecule, amyloid-beta (AB), all clinical trials have, so far, failed to demonstrate efficacy in restraining the severe, progressive, cognitive impairment and related symptoms in patients. We have demonstrated that the potent neuroinflammatory cytokine TRAIL, which belongs to the TNF superfamily, mediates and amplifies the neurotoxic effects of AB in vitro and in vivo, by recruitment of an array of proinflammatory factors and setting into motion of the apoptotic machinery. Interestingly, chronic intraperitoneal treatment with an anti-TRAIL monoclonal antibody, results in dramatic reduction of cognitive decline in 3xTg-AD mice.

In order to optimize anti-TRAIL administration and to obtain a more appropriate method for it to cross the blood-brain-barrier and reach the brain, we investigated the difference between both the free and the nanoparticle-conjugated forms of the anti-TRAIL antibody.

In preliminary studies aimed to compare the antiapoptotic effects of different formulations of the anti-TRAIL antibody, the effects of both preparations were evaluated in the macrophage cell line RAW incubated with TRAIL for 72 h, alone or in combination with either anti-TRAIL or with nanoparticles conjugated with the α-TRAIL antibody (NANO-A NANO-B). At the end of the experiments, cell viability was measured by the MTT assay.

Results showed that the TRAIL-neutralizing efficacy was similar for the two preparations, and in both cases the treatment totally prevented TRAIL-induced cell death in RAW cell cultures.

Finally, detection of different formulations of novel drugs which allow alternate routes of administration, appropriate to cross the blood brain barrier, thus being vehiculated directly to the brain, may represent a platform for innovative efficacious treatment of AD.

P 50/10

THE TRAIL/GITRL INTERPLAY IN NEURODEGENERATIVE PROCESSES IN THE HCN-2 HUMAN CORTICAL CELL LINE IN VITRO

Di Benedetto Giulia, Ronsisvalle Nicole, Bernardini Renato, Cantarella Giuseppina

Department of Biomedical and Biotechnological Sciences, University of Catania ~ Catania ~ Italy

Cytokines belonging to the TNF superfamily play a relevant role in neurodegenerative processes. Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), released during neuronal injury, has proven to potently mediate and sustain neurotoxic processes leading to neuronal death. Similarly to TRAIL, the cytokine Glucocorticoid-induced TNF receptor ligand (GITRL) is able to transduce proapoptotic signals. In spite of the array of reports suggesting relationships between TRAIL and other cytokines, scanty data are, so far, available about a TRAIL/GITRL crosstalk.

Here, we investigated possible interactions between TRAIL and the GITRL system in an in vitro model of neurodegeneration, using the human cortical neuronal cell line HCN-2.

Cultured HCN-2 neurons were incubated at different times with GITRL and/or TRAIL, and thereafter nucleic acid and protein expression were measured.

Real-time PCR analysis showed that the human cortical neuronal cell line HCN-2 do not express GITRL mRNA, but the latter is induced after treatment with TRAIL. In addition, HCN-2 cells did not express the GITRL receptor GITR mRNA, neither in control cultures, nor after treatment with TRAIL. All mRNA data were confirmed by western blot analysis of proteins. Cell viability assay showed that TRAIL, when associated to GITRL, was able to exert additive toxic effects. A counterproof was provided in experiments performed blocking GITRL, in which TRAIL-mediated toxicity appeared significantly reduced.

Results suggest that TRAIL/GITRL redundancy during neurodegenerative processes implies extended potentiation of detrimental effects of both cytokines on neurons, eventually leading to larger cell damage and death. Finally, characterization of novel molecular targets within the TRAIL/GITRL interplay may represent a platform for innovative therapy of neurodegenerative disorders.

P 51/10

FUNCTIONAL READOUTS OF NEURONS DIFFERENTIATED FROM HIPS DERIVED FROM PATIENTS AFFECTED BY HUNTINGTON'S DISEASE

Cesana Elisabetta[1], Conforti Paola[2], Cattaneo Elena[2], Toselli Mauro[1], Biella Gerardo[1]

[1]University of Pavia ~ Pavia ~ Italy, [2]University of Milan ~ Milan ~ Italy

Huntington's disease (HD) is a fatal neurodegenerative disorder correlated to an expanded CAG repeat within the IT-15 gene encoding for the protein huntingtin. HD is characterized by motor, cognitive and psychiatric symptoms and by a progressive atrophy and neuronal death of striatal medium-sized spiny neurons (MSNs) within the basal ganglia. Recently, the generation of human induced pluripotent stem cells (hiPS) has provided a powerful tool to reproduce in vitro models of human pathologies.

Here, we aim to show preliminary results from histochemical assays and electrophysiological recordings from monocultures of hiPS derived from fibroblasts of healthy subjects with 28 or 33 CAG repeats and HD patients with 60 or 109 CAG repeats.

Analysis were performed 30 days after the cells have been differentiated using an ontogeny-recapitulating protocol (Delli Carri et al., 2013), which drives hiPS to acquire a striatal fate and to develop the neuronal identity typical of authentic MSNs.

In this study the differences in the expression and in the biophysical properties of the voltage-dependent sodium, potassium and calcium channels among the two control and the two HD conditions were investigated; similarly the functionality of the main glutamatergic and GABAergic receptors were assessed.

These results support the idea of cell reprogramming as a promising strategy to model the HD and promote the use of neurons derived from hiPS in cellular therapy.

P 52/10

IN VIVO DOPAMINE AGONIST PROPERTIES OF ROTIGOTINE: ROLE OF D1 AND D2 RECEPTORS

Fenu Sandro[1], Espa Elena[1], Pisanu Augusta[2], Di Chiara Gaetano[1]

[1]Department of Biomedical Sciences University of Cagliari ~ Cagliari ~ Italy, [2]National Research Council of Italy, Neuroscience Institute, Cagliari Section ~ Cagliari ~ Italy

Rotigotine is a non-ergolinic dopamine receptor agonist, which acts in vitro as a full agonist of dopamine D_1 receptors at concentrations almost superimposable to those at which it acts at D_2 receptors. However in vivo evidence of D_1 agonist activity of rotigotine has not been yet provided.

In order to test the ability of rotigotine to stimulate dopamine D_1 and D_2 receptors, we studied the effect of selective dopamine D_1 and D_2/D_3 receptor antagonists on rotigotine-induced contralateral turning behavior in a rat model of Parkinson's disease, and the expression of the immediate-early gene c-fos in the caudate-putamen.

The effect of SCH 39166 (0.1 mg/kg s.c.) and eticlopride (0.1 mg/kg s.c.) was tested on rotigotine-induced contralateral turning behavior (0.035, 0.1 and 0.35 mg/kg s.c.), in drug naive or primed rats, after unilateral 6-hydroxydopamine lesion in the medial forebrain bundle. In the same model we tested the ability of rotigotine to stimulate the expression of the immediate-early gene c-fos in the caudate-putamen, an in vivo marker of D_1 receptor activity. As comparison we tested in parallel the D_2/D_3 agonist pramipexole.

In primed 6-hydroxydopamine lesioned rats, rotigotine induced a dose-dependent contralateral turning behavior. This effect was reduced by SCH 39166 and eticlopride only when rotigotine was administered at the intermediate dose (0.1 mg/kg s.c.). In drug-naive rats rotigotine was less effective in inducing contralateral turning and SCH 39166 reduced it. On the other hand, pramipexole induced contralateral turning in primed but not in drug-naive rats and this effect was potentiated by SCH 39166 and abolished by eticlopride pretreatment. Finally, rotigotine, but not pramipexole, induced Fos expression in the caudate-putamen at all tested doses, and this effect was abolished by SCH 39166 pretreatment.

These results show that rotigotine acts in vivo as a D_1/D_2 agonist, in contrast with pramipexole which is devoid of D_1 activity in vivo. These observations would predict that rotigotine and pramipexole might also differ in their spectrum of application to the therapy of Parkinson's disease.

P 53/10

WHITE MATTER DIFFUSION PARAMETERS IN MIGRAINE WITH AND WITHOUT AURA

Szabo Nikoletta[2], Kiraly Andras[3], Farago Peter[3], Csete Gergo[3], Toth Eszter[3], Kocsis Krisztian[3], Pardutz Arpad[3], Szok Delia[3], Tajti Janos[3], Vecsei Laszlo[1], Kincses Tamas Z [3]

[1]Department of Neurology, Albert Szent-Györgyi Clinical Center, University of Szeged / MTA-SZTE Neuroscience Research Group ~ Szeged ~ Hungary, [2]Department of Neurology, Albert Szent-Györgyi Clinical Center, University of Szeged / Central European Institute of Technology, Brno ~ Szeged / Brno ~ Czech Republic, [3]Department of Neurology, Albert Szent-Györgyi Clinical Center, University of Szeged ~ Szeged ~ Hungary

Disintegration of white matter in a mixed group of migraine patients with or without aura was found formerly (Szabó et al 2010), but it was not evident from our former results if the alterations were related to aura symptoms.

Here we aimed to explore white matter alterations in a homogeneous group of patients with migraine with aura and to delineate possible relationships between white matter changes and clinical variables.

Eighteen patients with aura (MwA), thirty-five migraine patients without aura (MwoA) and thirty-two controls were scanned on a 1.5T MRI scanner. Diffusivity parameters of the white matter were estimated and compared between patients' groups and controls using a whole-brain tract based spatial statistics. Correlation analysis was conducted between diffusivity parameters from the altered regions and clinical variables.

Widespread increase of fractional anisotropy (FA) ($p<0.007$), decrease of mean (MD) ($p < 0.007$) and radial diffusivity (RD) ($p<0.006$) was found in the white matter of MwA patients compared to controls. The diffusivity parameters of MwoA patients showed no alteration compared to controls. Decreased FA ($p<0.048$) was found in MWA compared to MwoA in the left parieto-occipital white matter. FA showed negative ($p<0.044$, $R = 0.480$), MD ($p<0.031$, $R = 0.508$) and RD ($p < 0.030$, $R = 0.511$) showed positive correlation with the attack duration in the MwA patients. Subjective pain intensity showed positive correlation with MD ($p<0.039$, $R = 0.489$) and RD ($p<0.044$, $R = 0.480$). The diffusion parameters did not correlate with allodynia score, disease duration, age and attack frequency.

These results in comparison with our former results show that migraine is a heterogeneous disease. We propose that degenerative and maladaptive plastic changes coexist in the disease and the diffusion profile is a resultant of these processes. And we might consider the possibility that MwA is a separate headache disorder with different pathomechanism, but at least it is better to investigate to two forms separately.

P 54/10

NCX1 EXCHANGER COOPERATES WITH CALRETININ TO CONFER PRECONDITIONING-INDUCED TOLERANCE AGAINST CEREBRAL ISCHEMIA IN THE STRIATUM

De Rosa Valeria, Boscia Francesca, Casamassa Antonella, Secondo Agnese, Esposito Alba, Pannaccione Anna, Sirabella Rossana, Pignataro Giuseppe, Cuomo Ornella, Vinciguerra Antonio, Annunziato Lucio

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, "Federico II" University of Naples ~ Naples ~ Italy

Recently, the Na^+/Ca^{2+} exchanger NCX1 and the calcium-binding protein calretinin, have emerged as new molecular effectors of delayed preconditioning in the brain.

In the present study, we investigated whether NCX1 and calretinin cooperate within the preconditioned striatum to confer neurons greater resistance to degeneration.

To this aim biochemical and confocal double immunofluorescence experiments were applied to both in vitro and in vivo models of ischemic preconditioning to analyze the distribution and coexpression of NCX1 with calretinin in brain cells. Furthermore, gene silencing strategy was used to investigate the cooperation between NCX1 and calretinin during ischemic preconditioning.

Confocal microscopy analysis revealed that NCX1 expression was upregulated in calretinin-positive interneurons in the rat striatum after tolerance induction. Consistently, co-immunoprecipitation assays performed on human SHSY-5Y cells, a neuronal cell line which constitutively expresses calretinin, revealed a binding between NCX1 and calretinin. Finally, silencing of calretinin expression, both in vitro and in vivo, significantly prevented preconditioning-induced neuroprotection. Interestingly, our biochemical and functional studies showed that the selective silencing of calretinin in brain cells significantly prevented not only the preconditioning-induced upregulation of NCX1 expression and activity, but also the activation of the prosurvival protein kinase Akt, which is involved in calretinin and NCX1 protective actions.

Collectively, our results indicate that the Na^+/Ca^{2+} exchanger NCX1 and the calcium-binding protein calretinin cooperate within the striatum to confer tolerance against cerebral ischemia.

P 55/10

EARLY STAGE DETECTION OF ALPHA SYNUCLEIN ACCUMULATION INDUCED CHANGES IN TRANSGENIC MOUSE MODEL OF PARKINSON DISEASE: A DIFFUSION KURTOSIS IMAGING STUDY

Arab Anas[1], Khairnar Amit[2], Kucerova Jana[3], Latta Peter[4], Szabo Nikoletta[5]

[1]Experimental and Applied Neuropsychopharmacology Group, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic ~ Brno ~ Czech Republic, [2]Applied Neuroscience Research Group, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic. ~ Brno ~ Czech Republic, [3]Masaryk University, Faculty of Medicine, Department of Pharmacology, Brno, Czech Republic. ~ Brno ~ Czech Republic, [4]Multimodal and Functional Imaging Laboratory, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic. ~ Brno ~ Czech Republic, [5]Department of Neurology, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary ~ Szeged ~ Hungary

One of the challenges in Parkinson's disease (PD) is to find an imaging biomarker which could detect PD at early stages of the pathology, e.g. alpha-synuclein accumulation. Diffusion kurtosis imaging (DKI) technique is one of the diffusion weighed methods which was proved to assess non-gaussian water diffusion. This measure is known to be able to detect microstructural changes in neurodegenerative diseases, e.g. in Alzheimer's disease.

The aim of this study was to assess whether DKI method is able to detect early alpha-synuclein accumulation in substantia nigra, striatum, hippocampus and thalamus in a transgenic mouse model of PD thy1-asynuclein mice of line 61 (TNWT-61) and confirm the PD-like phenotype with behavioral profile for motor impairment. We hypothesized that presence of alpha-synuclein accumulation would create more water diffusion barriers resulting in higher kurtosis values in the MRI study and the animals would already show significant motor impairment.

Three month old TNWT-61 mice and wild-type (WT) littermates underwent grid test, beam walk (square and round) and challenging beam walk tests to assess motor impairment and MRI scanning using 9.4 Tesla system in vivo. DKI maps (mean, axial and radial kurtosis, mean, axial and radial diffusivity and fractional anisotropy) were obtained for substantia nigra, striatum, hippocampus and thalamus using Explore DTI® software. We chose these specific regions of interest (ROIs) based on the substantial accumulation of alpha-synuclein found previously in TNWT-61 mice. The ROIs were drawn manually according to the Paxinos Mouse Brain Atlas (2001) with the help of fractional anisotropy maps using ImageJ® software.

We found significant increases in mean kurtosis in striatum and thalamus in the TNWT-61 mice. The other parameters did not show significant changes. This is consistent with our previous studies in older TNWT-61 animals where the kurtosis changes were detected in more ROIs. Furthermore, the transgenic mice made significantly more slips and needed more time to traverse the beam than the WT littermates in all beam tests. Latency to fall in the grid test was significantly shorter in this group as well.

The current study provides evidence that DKI is sensitive for a very early in vivo detection of pathological changes that underlie PD-like symptomatology in the TNWT-61 mouse model of PD.Striatum and thalamus seem to be the most sensitive ROIs for an early detection of the pathology in this model.

The project is financed from the SoMoPro II Programme, co-financed by the European Union and the South-Moravian Region.

P 56/10

ON THE USE OF THE POSITIVE AND NEGATIVE SYNDROME SCALE IN RANDOMIZED CLINICAL TRIALS

Nicotra Eraldo[1], Casu Gianluca[2], Piras Sara[2], Palomba Mario[2], Marchese Giorgio[2]

[1]Department of Pedagogy, Psychology, Philosophy, Faculty of Humanities, University of Cagliari ~ Cagliari ~ Italy, [2]National Research Council, Institute of Translational Pharmacology, UOS of Cagliari ~ Pula ~ Italy

In the last 25 years, the Positive and Negative Syndrome Scale (PANSS) has been largely used to assess schizophrenia symptom intensity, but little information is available on how this scale was generally applied when evaluating the efficacy of new therapies in randomized clinical trials.

The study focused on (i) how PANSS results are depicted within research articles; (ii) the PANSS-subscale structures mostly used in randomized clinical trials; (iii) the demographic and symptomatic characteristics of schizophrenic individuals enrolled in randomized clinical trials.

A systematic PubMed Search was carried out using the keywords “PANSS” and “Randomized Clinical Trials”.

The analysis of retrieved articles confirmed that PANSS constitutes a valuable psychometric instrument to investigate the effects induced by pharmacological and non-pharmacological therapies in schizophrenia individuals. However, the information potentially provided by this scale was only partially reported in research articles, when characterizing the symptomatic features of patients at baseline. Furthermore, the rationale behind the use of a specific PANSS-subscale structure was rarely mentioned in PANSS-RT articles, and the choice appeared to be mostly related to the trial sample size or geographic/cultural factors. Unexpectedly, it was estimated that 95% of schizophrenic individuals enrolled in randomized clinical trials showed a symptom intensity ranging from “minimal” to “moderate” both in PANSS total and PANSS subscales.

A further effort is needed to increase the amount of qualitative and quantitative information potentially provided by PANSS within research articles, since a more complete description of the symptomatic characteristics of patients enrolled in randomized clinical trials may improve the transferability of the results to the clinical practice and drug development research. Possibly, the completeness of PANSS-subscale scores and a larger use of factorial analyses may provide useful data for reaching a consensus on which PANSS-subscale structure better represents the symptomatic profiles of schizophrenic individuals. The possibility that the structured PANSS interview may limit the enrollment of schizophrenic individuals with “severe” symptoms should be carefully analyzed, so to avoid a possible gap between the results of clinical trials and the everyday clinical practice.

P 57/10

EFFECTS PRODUCED BY M2 MUSCARINIC RECEPTOR ACTIVATION IN BRAIN TUMORS: POSSIBLE THERAPEUTIC IMPLICATIONS

Cristofaro Ilaria[1], Mattei Francesca[1], Alessandrini Francesco[1], Spinello Zaira[1], Di Bari Maria[1], Orlando Viviana[1], Conti Luciano[2], Tata Ada Maria[1]

[1]Dept. of Biology and Biotechnologies Charles Darwin, Sapienza Università di Roma, Research Center of Neurobiology Daniel Bovet ~ Roma ~ Italy, [2]Centre for Integrative Biology (CIBIO), University of Trento ~ Trento ~ Italy

Muscarinic acetylcholine receptors (mAChRs) are expressed in several primary and metastatic tumours such as colon, ovary, prostate, lung carcinomas, breast cancer and melanoma. In some cases ACh, synthesized by the tumour cells, modulates cell proliferation by an autocrine mechanism involving cholinergic receptors of nicotinic and muscarinic types. In the previous studies we demonstrated that the selective activation of M2 receptors in glioblastoma established cell lines caused a decreased cell proliferation and enhanced cell death.

In the present study we evaluated the ability of M2 receptors to modulate cell growth, cell death, and the mechanism downstream their selective activation in glioblastoma cancer stem cells and in neuroblastoma cell lines.

FACS analysis was used to evaluate the cell cycle progression and levels of apoptosis after M2 agonist treatment in glioblastoma cancer stem cells (GSCs) and in neuroblastoma cell cultures. The cross talk between M2 receptor and Notch and EGFR pathway was analyzed by RT-PCR and western blot analysis. Pharmacological competition experiments and siRNA transfection for M2 receptors were used to confirm the selective involvement of M2 receptor.

TheM2 agonist arecaidine propargyl ester (APE) was used to induce the selective activation of M2 receptor. The stimulation of this receptor caused a decrease of cell proliferation in particular in one GSC cell line (GB7) characterized by higher expression of M2 receptors compared with the other GSC cell (GB8) in which APE induced a severe apoptosis. The anti-proliferative effects of M2 antagonist appeared mainly dependent on the decreased Notch and EGFR expression-APE induced. The use of specific muscarinic antagonists and the silencing of the M2 receptor have demonstrated the direct involvement of the M2 receptor in the inhibition of glioma cell proliferation. Similarly, in Neuroblastoma cell lines (SK-N-SH, SK-N-MC), APE was able to inhibit cell proliferation without affecting cell viability. Interestingly APE was also able to decrease c-myc expression and reduce the Notch and EGFR expression-APE induced.

The data obtained suggest that the M2 receptors may be a new interesting therapeutic tool in the treatment of glioblastomas and neuroblastomas. Therefore, the identification of new agonists selective for these receptors may acquire a clinical relevance in brain tumor therapy.

P 58/10

NEUROPROTECTIVE AND ANTI-INFLAMMATORY PROPERTIES OF MDG548, A NOVEL PPARG AGONIST, IN CHRONIC MPTPP MODEL OF PARKINSON'S DISEASE

Lecca Daniela, Mulas Giovanna, Diana Andrea, Carta Anna Rosa

Dept. Biomedical Sciences, Univ. of Cagliari ~ Cagliari ~ Italy

Peroxisome proliferator activated receptor (PPAR)γ, with its efficacy on modulation of neuroinflammatory response, is a potential pharmacological target in Parkinson's disease (PD). However, currently available PPARγ agonists thiazolidinediones (TZDs) present limitations due to safety concerns: rosiglitazone has been related with negative effects on cardiovascular function and pioglitazone with an increased bladder cancer risk. This limitation has prompted for the search of novel non-TZD compounds devoid of side-effects.

We tested the novel PPARγ agonist MDG548, which shows high binding affinity and selectivity, by in vitro experiments and in a chronic MPTP mouse model, aimed at investigating neuroprotective properties in PD.

For in vitro studies, neutral red uptake assay for cell viability was used to test neuroprotective properties of MDG548 against MPP^+ toxicity in PC12 dopamine-like cells. For in vivo studies, C57BL/6J mice received chronic MPTP (25 mg/kg i.p) plus probenecid (100 mg/kg i.p) twice a week for 5 weeks, in association with saline or MDG548 (2 mg/kg i.p.) in the last week of MPTP treatment. Motor behavior was evaluated by the beam test after completion of treatments. Coronal sections from the substantia nigra pars compacta (SNc) were immunoreacted against tyrosine hydroxylase (TH), CD11b and TNF-alpha.

In vitro studies showed that MDG548 prevented MPP^+-induced cell death of PC12 cells. In vivo, the beam walking test showed that chronic MPTP treatment produced a significant increase in the number of stepping errors as compared to vehicle, which was reverted by combined treatment with MDG548. Stereological counting showed that MDG548 prevented the MPTP-induced reduction in TH-positive cells in the SNc. Moreover, evaluation of the inflammatory response showed that CD11b(+) microglia and TNF-alpha-IR colocalization with microglia were increased by chronic MPTP treatment as compared to vehicle in the SNc, while they were significantly reduced by MDG548 co-treatment.

MDG548, considering both the neuroprotective and anti-inflammatory properties in PD models, might be a promising alternative in the search for potent PPARγ agonists to be tested as disease-modifying drugs in PD.

P 59/10

METFORMIN REVERSES THE DOPAMINERGIC NEUROTOXICITY INDUCED BY REPEATED 3,4-METHYLENEDIOXYMETHAMPHETAMINE ADMINISTRATIONS

Porceddu Pier Francesca[1], Ishola Ismail[2], Morelli Micaela[1]

[1]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos ~ Lagos ~ Nigeria

Metformin, a well known antidiabetic drug, has been recently investigated and proposed to promote neurogenesis and, especially, to be protective in neurodegenerative processes induced by the dopaminergic neurotoxin, MPTP, model of Parkinson's Disease. Elevated levels of glia-generated reactive species such as cytokines, have been shown to correlate with neurodegeneration induced by amphetamine-related drugs. Interestingly, metformin is involved in regulating the production of cytokines, released during a neuroinflammatory process and through the up-regulation of the anti-inflammatory cytokines release, and an antioxidant activity, attenuates the neurotoxic properties of microglia.
3,4-Methylenedioxymethamphetamine (MDMA) is a recreational drug mostly consumed by young adults in raves and disco clubs. Several studies have reported neurotoxic and neuroinflammatory properties of MDMA; in mice, MDMA produces a persistent loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and in caudate putamen (CPu).

On these basis the aim of this study was to investigate the neuroprotective effect of metformin against MDMA-induced neurotoxicity.

We evaluated the neuroprotective effect of metformin (2×200 mg/kg) on dopaminergic MDMA-induced damage by tyrosine hydroxylase (TH) immunohistochemistry in SNc and CPu of adult mice treated with repeated MDMA (4×20 mg/kg) administrations.

The metformin treatment was able to prevent the decrease of TH-positive neurons in the SNc, and the TH-positive fibers in CPu, induced by MDMA.

Metformin showed a neuroprotective efficacy on the neurodegenerative effects of repeated MDMA administrations, confirming the therapeutic potential of this compound on neurodegenerative process.

P 60/10

THE PHARMACOLOGICAL PROFILE OF THE DUAL RECEPTOR GPR17

Marucci Gabriella[1], Buccioni Michela[1], Dal Ben Diego[1], Lambertucci Catia[1], Santinelli Claudia[1], Spinaci Andrea[1], Ajiroghene Thomas [1], Abbracchio Maria Pia[2], Volpini Rosaria[1]

[1]University of Camerino ~ Camerino ~ Italy, [2]University of Milano ~ Milano ~ Italy

Purinergic signalling is now recognized to be involved in a wide range of activities: extracellular adenine and uracil nucleotides mediate short-term (acute) signalling functions in neurotransmission, secretion and vasodilatation, and long-term (chronic) signalling functions in development, regeneration, proliferation and cell death. GPR17 is a peculiar Gi protein-coupled receptor showing a dual pharmacological profile. In fact, it seems to be specifically activated by two unrelated families of inflammatory mediators: extracellular nucleotides and cysteinyl-leukotrienes, which are massively released upon different types of injury. In facy, it is pathologically activated during acute CNS injury, thus contributing to early necrotic death inside the lesion, but subsequently after the damage, it seems to promote cell repair mechanisms by activation/proliferation of reactive astrocytes and oligodendrocyte precursor cells (OPCs). Hence, it may represent a novel target for human neurodegenerative diseases like stroke, trauma, and multiple sclerosis. The cysteinyl-leukotriene LTD4 (EC50 3.15 nM) and the purinergic mediator UDP (EC50 1,779 nM), together with other synthetic purine nucleotide derivatives, activate GPR17 with EC50 of 3.15 nM and 1,779 nM, respectively, leading to both adenylyl cyclase inhibition and intracellular calcium decrease.

In order to pharmacologically characterize the dual profile of GPR17, a new compound designed and synthesized to be able to interact with both the binding sites present in the receptor was tested in comparison with known nucleotides and cysteinyl-leukotriene agonists and antagonists.

The experiments were performed using the GloSensor™ cAMP assay, that allows to monitor GPCR activity through change in the intracellular cAMP concentration. Human embryonic kidney (HEK293) L9-2 cells, stably transfected with the biosensor and transiently with human GPR17 receptor, were used to detect the activity of compounds under study.

Results obtained with different concentrations of the new ligand co-incubated with reference antagonists, montelukast and PF4, showed that the designed compound is really able to interact with both sites expressed in the receptor.

These resultes show that the new compound behaves as a dual ligand of GPR17.

P 61/10

A COMPREHENSIVE STUDY ON OLEUROPEIN AGLYCONE BENEFICIAL EFFECTS IN THE TGCRND8 MOUSE MODEL OF AB DEPOSITION: A MECHANISTIC INSIGHT

Pantano Daniela[1], Nardiello Pamela[2], Cavone Leonardo[1], Lapucci Andrea[1], Luccarini Ilaria[1], Stefani Massimo[2], Casamenti Fiorella[1]

[1]Department of Neuroscience, Psychology, Drug Research and Child Health, Division of Pharmacology and Toxicology ~ Florence ~ Italy, [2]Dept. Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy ~ Florence ~ Italy

The Mediterranean diet is claimed to reduce age-related dysfunctions including Alzheimer's disease (AD) possibly due to the presence of substantial amounts of polyphenols. Oleuropein and its aglycone (OLE) are the major polyphenols in the extra virgin olives oil.

In TgCRND8 mice and w.t. control littermates of different ages, from pre-Aβ to the late stage of Aβ deposits fed or not for 8 weeks with different amounts of OLE (0.5, 12.5, 50 mg/kg of diet) (8-10 mice/group) we investigated the effects of OLE on cognitive functions, on the glutaminyl cyclase (QC)-produced pE(3-42)Aβ plaques and QC activity/expression and synaptic morphology and function.

The step-down inhibitory passive avoidance and object recognition tests were employed to evaluate the cognitive functions; immunohistochemical analysis and Western blotting were used to detect pE(3-42)Aβ plaques and QC activity/expression and LTP in the CA1 hippocampal area was performed to analyze the synaptic plastcity.

We found that OLE (50 mg/kg of diet) supplementation with diet significantly protects against cognitive deterioration, remarkably reduces β-amyloid levels and plaque deposits which appeared less compact and ''fluffy'', is active against QC-catalyzed pE3-Aß generation reducing enzyme expression and interferes with both Aβ42 and pE3-Aβ aggregation. Moreover, the phenol activates neuronal autophagy via the AMPK/mTOR signaling pathway even in mice at advanced stage of pathology, where it increases histone 3 and 4 acetylation, which matches both a decrease of histone deacetylase 2 expression and a significant improvement of synaptic function. Finally, in the brain of OLE-fed TgCRND8 animals we found that the phenol significantly reduced poly(ADP-ribose) polymerase-1 (PARP-1) activity leading to a reduction in PAR polymers.

The occurrence of these functional, epigenetic and histopathological beneficial effects even at a late stage of the pathology suggests that the phenol could be beneficial at the therapeutic, in addition to the prevention, level. In conclusion our results agree with those reported for other plant polyphenols, suggesting a shared molecular mechanism underlying the health effects of these substances against ageing and neurodegeneration. The dose-response evaluation of OLE on cognitive functions and neuropathology is undergoing.

P 62/10

TIME-COURSE OF THE PROTECTIVE EFFECT OF SELECTIVE ADENOSINE A_{2A} RECEPTOR AGONISTS AND ANTAGONISTS AFTER BRAIN FOCAL ISCHEMIA IN THE RAT.

Dettori Ilaria, Melani Alessia, Cellai Lucrezia, Pedata Felicita

Dept. of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Italy ~ Florence ~ Italy

In recent years, evidence indicated that adenosine A_{2A} receptor subtype is of critical importance in stroke. Adenosine A_{2A} receptors located on neuronal and microglial cells of striatum and cortex are overexpressed 24 hours after focal cerebral ischemia.

Aims of the work were to investigate the protective effect of the adenosine A_{2A} receptor agonist, CGS 21680, and of the adenosine A_{2A} receptor antagonist, SCH 58261, chronically administered (0.01 mg/kg, i.p., twice/day for 7 days) after transient (1 hour) focal ischemia induced in the rat by occlusion of the middle cerebral artery (tMCAo).

The protective effects of the adenosine A_{2A} receptor agonist,CGS 21680 and of the adenosine A_{2A} receptor antagonist, SCH 58261 were evaluated by neurological tests and by immunohistochemical analysis.

CGS 21680, administered starting from 4 hours after ischemia, protected from neurological deficit from the first day up to seven days thereafter (score at 7 day: 4.37 ± 0.90, n = 4 versus 7.00 ± 0.64, n = 9 in vehicle group; $p<0.001$). Seven days after the ischemic insult, it significantly reduced the volume of the ischemic cortical damage (51.88 ± 10.37 mm^3, n = 4 versus 75.15 ± 5.13 mm^3, n = 9 in vehicle group; $p<0.02$), improved the cytoarchitecture of ischemic cortex and striatum, improved the myelin organization in ischemic striatum, reduced microgliosis and astrogliosis. Two days after tMCAo, a massive infiltration of granulocytes into cerebral ischemic tissue was observed. CGS 21680 reduced granulocyte infiltration in the ischemic areas (in cortex: 20.32 ± 2.41 cells/optical field, n = 6 versus 37.5 ± 6.8, n = 3 in vehicle group; $p < 0.02$). SCH58261, administered starting from 5 min after ischemia significantly protected from the neurological deficit 1 day after tMCAo ($p<0.001$), but no more after 5 and 7 days. Seven days after tMCAo, SCH 58261 has not protected ischemic areas from damage and has not ameliorated myelin organization into the ischemic striatum. Two days after tMCAo, SCH 58261 has not reduced blood cell infiltration into ischemic striatal and cortical tissue.

Results indicate that CGS 21680, chronically administered, protects against ischemic damage by reducing blood cell infiltration and neuroinflammation along the days after ischemia. Moreover, protection by SCH 58261 24 hours after ischemia is attributable to reduced excitotoxicity. Seven days after ischemia the early protective effect of the A_{2A} receptor antagonist, likely has been overwhelmed by a secondary damage due to blood cell infiltration and neuroinflammation.

P 63/10

ALTERED ADULT NEUROGENESIS AND ENHANCED SEIZURE PROPENSITY IN OLIGOPHRENIN-1 KNOCK-OUT MICE, A MURINE MODEL OF X-LINKED INTELLECTUAL DISABILITY

Allegra Manuela[1], Spalletti Cristina[1], Vignoli Beatrice[2], Azzimondi Stefano[1], Busti Irene[1], Canossa Marco[2], Caleo Matteo[1]

[1]Neuroscience CNR ~ Pisa ~ Italy, [2]University of Bologna ~ Bologna ~ Italy

Oligophrenin-1 (OPHN1) gene is located on the X chromosome, whose mutations cause X-linked intellectual disability and epilepsy. OPHN1 encodes a RhoGTPase-activating protein that regulates neuronal morphology, cell proliferation and migration, and participates in synaptic function. OPHN1 knock out (KO) mice exhibit impairments in spatial memory and social behavior, an immature phenotype of dendritic spines in CA1 pyramidal neurons associated with altered synaptic plasticity. However, how mutations in OPHN1 affect circuit formation, with consequent cognitive impairment and network hyperexcitability remains still incompletely understood.

To investigate adult hippocampal neurogenesis and seizure propensity in OPHN1 KO vs. wt animals.

We used labeling of newborn hippocampal neurons with either bromodeoxyuridine or retroviral vectors expressing GFP. For the evaluation of seizure propensity, we performed local field potential (LFP) recordings from the hippocampus in freely moving mice.

We found significant impairments in differentiation, migration and integration of newborn neurons in the dentate gyrus of OPHN1 KO mice as compared to controls. We found an altered morphological maturation of newly generated cells, in particular a lack of axonal extension towards the CA3 area and reduced neuronal survival. Some of these pathological phenotypes could be rescued by systemic administration of fasudil, an inhibitor of the Rho kinase (ROCK) whose activity is potentially up-regulated by loss of OPHN1. We also investigated seizure propensity in KO vs. wt animals. By in vivo recordings of hippocampal activity in freely moving animals (two months of age), we found significant epileptiform alterations in OPHN1 KO but not wt mice. We also studied seizure activity induced by administration of the glutamatergic agonist kainic acid, and we found higher susceptibility to seizures in OPHN1 KO mice. This was accompanied by neuropathological alterations such as GABAergic interneuronal death and neuropeptide Y (NPY) upregulation.

Altogether, our results demonstrate that loss of function of OPHN1 affects adult hippocampal circuitry, causing alterations in neurogenesis and enhanced seizure susceptibility.

P 64/10

OREXINERGIC NEURONS IN MURINE MODELS OF ALZHEIMER'S DISEASE

Andrioli Anna[1], Wirtu Amenu Tolera[1], Azeez Idris[1], Dix Sophie[3], Richardson Jill C[2], Bentivoglio Marina[1]

[1]Dept of Neurological and Movement Sciences, University of Verona ~ Verona ~ Italy, [2]Neuroscience Therapeutic Area, GlaxoSmithkline R&D ~ Stevenage ~ United Kingdom, [3]Lilly, Lilly Research Centre ~ Windlesham, Surrey ~ United Kingdom

The pathological hallmarks of Alzheimer's disease (AD), the most common form of dementia, are notably represented by extracellular plaques of beta-amyloid protein (Aβ), intracellular neurofibrillary tangles and neuroinflammation. Clinically, AD leads to impairment of cognitive abilities, severe memory loss, and sleep dysregulation. Recently, sleep dysregulation has been proposed to contribute to Aβ pathology and to exacerbate the pathological features of AD. In the sleep-wake regulatory networks, neurons of the lateral hypothalamus which contain the hypocretin/orexin (OX) neuropeptides have attracted considerable interest for their role in arousal, wake stability, energy homeostasis and motivated behavior. Post-mortem examination of AD brains has revealed loss (about 40%) of orexinergic neurons. The potential role of OX-A level in the cerebrospinal fluid as AD biomarker during disease progression is currently highly debated.

The present study was undertaken to examine the number of orexinergic neurons and OX-A expression in three transgenic (Tg) murine models of AD, comparing mice before the onset of the pathology with aged animals showing pathological AD hallmarks.

The study was based on single mutant PDAPP mice of 6 and 24 months of age; double mutant TASTPM mice of 3, 15 and 18 months of age; triple mutant B6 TAU 152 (3XTg) mice of 8 and 24 months of age. All of the Tg mouse groups were compared with age-matched wild-type (WT) littermates. OX-A was visualized by immunohistochemistry. The number of OX-A-containing neurons was evaluated with unbiased stereological cell counts. Optical density (OD) quantification of OX-A expression was also pursued in 15 month-old TASTPM mice.

Qualitative observations showed a marked reduction of immunostained dendritic arborizations and shrinkage of some OX-A neurons in the older age groups of all genotypes, which were especially marked in the AD Tg mice. The quantitative evaluation showed that the aging-related reduction of orexinergic neurons documented in WT mice (about 20% decrease) was significantly exacerbated in the TASTPM mice (about 36% decrease) and 3XTg mice (about 40% decrease) of the older age groups. In addition, in the 15 month-old TASTPM mice the densitometric analysis revealed in the orexinergic cell bodies a significant upregulation of OX-A expression with respect to the matched WT mice.

The findings show that the pathological process in murine AD models targets orexinergic neurons during disease progression. The data also indicate that in the cells in which OX-A immunostaining is preserved the production of peptide may increase, possibly as compensatory mechanism.

P 65/10

NEW THERAPEUTIC APPROACH FOR NEUROPATHIC PAIN: CELL AUTOPHAGY MODULATION VIA ENERGY BALANCE REGULATION

De Angelis Federica, Vacca Valentina, Nazio Francesca, Marinelli Sara

Santa Lucia Foundation ~ ROMA ~ Italy

After peripheral nerve injury, Schwann cells (SCs) degrade their own myelin and activate autophagy. This cytoprotective process is fundamental to remove myelin debris, and to promote axonal regeneration. When nerve insult and axon degeneration start, neuropathic pain (NeP) arises. Autophagy can be activated both pharmacologically and by metabolic signals. Nutritional triggers, such as starvation, are transduced via mammalian target of Rapamycin (mTOR), the switch of autophagic pathway, and AMP-activated protein kinase. AMBRA1 is thus one of functional targets of mTOR and one of the most upstream autophagy signalers. We previously demonstrated that in Ambra1+/gt mice, autophagy deficiency induced NeP exacerbation and in CD1 mice, rapamycin induced long-lasting analgesic and anti-inflammatory effects, facilitated nerve regeneration and prevented NeP chronification. On the other hand, we show here that both wild-type (WT) and Ambra1+/gt mice, subjected to caloric restriction (CR), restore normal pain perception and accelerate recovery from neuropathy. CR induces enhancement of autophagic flux in SCs, has anti-inflammatory property and ameliorates remyelization.

The potential benefits of CR on PNS are poorly examined and the study of CR-induced autophagy in NeP has never been investigated. The first objective aims to know if CR can have the same beneficial effect of rapamycin on allodynia and on SCs autophagic response. The second aim was to understand if CR dietary regimen can re-establish a balance between pro- and anti-inflammatory mediators in mice that underwent constriction injury of sciatic nerve (CCI). In response to nerve tissue damage, activation of immune cells leads to the production of inflammatory mediators, such as cytokines. At last we investigated if CR was able to facilitate regeneration and remyelization.

CD1/WT and Ambra1+/gt mice were subjected to CCI and randomly assigned to standard diet (ST) or CR group. In CR condition food intake was reduced of 40% for one week. Allodynia was evaluated throughout Dynamic Plantar Aesthesiometer Test. The autophagic process, remyelization and regeneration were examined by WB, immunofluorescence and confocal analysis in control and CCI nerves. Cytokines variation was evaluated in blood samples.

CR diet was able to dramatically anticipate NeP recovery even better than rapamycin, to increase autophagic flux in SCs and to accelerate myelin regeneration mainly in WT but also in Ambra1+/gt mice. Moreover, CR modulated both pro- and anti-inflammatory cytokines expression.

We demonstrate for the first time that dietary interventions are attractive, safe and inexpensive therapeutic options in NeP treatment.

P 66/10

CHOLINERGIC SYSTEM ALTERATIONS IN MULTIPLE SCLEROSIS: STUDIES IN RR-MS PATIENTS AND IN EAE MODEL

Di Bari Maria[1], Di Pinto Giovanni[1], Sperduti Samanta[2], Galizia Sabrina[1], Alvarez Rocio[3], Di Nicola Marta[4], D''Angelo Chiara[4], Orlando Viviana[1], Mengod Guadalupe[3], Gatta Valentina[2], Reale Marcella[4], Tata Ada Maria[1]

[1]Dept. Biol. Biotech. C. Darwin, Sapienza, University of Rome, Italy ~ Roma ~ Italy, [2]Dept. of Psychological, Health and Territorial Sciences, School of Medicine and Health Sciences, University " G. D'Annunzio" Chieti-Pescara ~ Chieti ~ Italy, [3]IIBB, CSIC, IDIBAPS, CIBERNED, Barcelona, Spain ~ Barcelona ~ Spain, [4]Dept. Medical, Oral and Biotech Science, University " G. D'Annunzio" Chieti-Pescara ~ Chieti ~ Italy

Acetylcholine (ACh) modulates the immune system and inflammation by a mechanism identified as "non-neuronal cholinergic anti-inflammatory pathway". Muscarinic and nicotinic receptors expressed by immune cells differently modulate the inflammatory mediators.

To evaluate whether inflammatory state in MS may be related to cholinergic system dysfunction we measured ACh levels, AChE and BuChE activity and expression and pro-inflammatory cytokines production in PBMCs and serum of RR-MS patients and healthy donors (HD). Moreover we evaluated the expression of cholinergic markers in brain and spinal cord of EAE mice.

ACh and cytokine levels were measured in serum and AChE and BuChE activities by Ellman test. qRT-PCR was performed to determine AChE, BuChE and cytokines mRNA expression. In situ hybridization was used to analyze ChAT, AChE and nicotinic alpha-7 receptor expression in EAE brain and spinal cord. Enzymatic histochemistry techniques and immunolocalization were used to correlate the expression of AChE and BuChE with glial markers.

Lower levels of ACh and higher levels of AChE and BuChE were observed in MS patients than in HD. PHA-stimulated expression of IL-1beta and IL-17 was significantly higher in PBMC of MS patients compared to HD. In PBMCs of MS patients, PHA plus nicotine co-treatment decreased the expression and production of these cytokines. Interestingly alpha-7 nAChR expression in PBMCs of MS patients was higher compared to HD with increasing levels after PHA stimulation. In CNS of EAE mice, ChAT mRNA levels increased in CNS cholinergic areas when compared to control CFA injected animals, whereas AChE and BuChE expression and enzymatic activity decreased. The expression of BuChE increased in glial cells.

Our results suggest that the decreased levels of ACh in MS may contribute to exacerbate the inflammatory state in MS. Reestablishment of cholinergic function may contribute to reduce the inflammatory state both in immune system and brain.

P 67/10

ROLE OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE IN (NEURO)INFLAMMATION

Pontis Silvia, Capurro Valeria, Summa Maria, Realin Natalia, Ribeiro Alison, Gujarro Ana, Armirotti Andrea, Reggiani Angelo, Piomelli Daniele[2]

[1]Drug Discovery Department, Fondazione Istituto Italiano di Tecnologia, via Morego 30, 16160 Genova, [2]Departments of Anatomy and Neurobiology, Pharmacology and Biological Chemistry, University of California, Irvine, CA 92697, USA

Multiple sclerosis (MS) is a chronic progressive disease of the central nervous system (CNS) characterized by autoimmune and aberrant inflammatory responses. Histologic examination of bioptic samples reveals foci of severe demyelination, decreased axonal and oligodendrocyte numbers, and glial scars. Preclinical and clinical studies showed that N-palmitoylethanolamide (PEA), a naturally occurring lipid amide, exerts anti-inflammatory, analgesic and neuroprotective effects. PEA inhibits mast cells degranulation and glia activation; its levels change during CNS pathological conditions affecting the progression of the neuroinflammatory process. Plasma and cerebrospinal fluid levels of NAEs are altered in MS patients. PEA is preferentially degraded by the N-acylethanolamine acid amidase (NAAA), we have previously demonstrated that NAAA inhibition normalizes PEA levels in several inflammatory models.

In order to investigate the role of NAAA in MS we induced the EAE model of MS in C57BL6/J mice and examined the expression of NAAA and inflammatory markers.

EAE was induced in mice by immunization with myelin oligodendrocyte peptide (MOG) 35-55 peptide, 200 µg/mouse. Mice also received intraperitoneal injections of 200 ng pertussis toxin (PTX) in 200 µL PBS after MOG injection and 48 h later. Control-immunized mice (injected with both MOG vehicle and PTX in the absence of MOG) in addition to unimmunized mice served as negative controls. Mice were weighted and scored daily for clinical signs as described by Stromnes and Goverman.

Analysis of qPCR data showed that NAAA and iNOS levels are significantly up regulated in mice showing clinical signs of EAE. Immunofluorescence analysis demonstrated that NAAA is upregulated in activated microglial cells. To determine whether NAAA upregulation in microglia cells affects the onset and progression of EAE we generated transgenic mice overexpressing NAAA (NAAA ki) in CD11b-positive cells. NAAA ki were obtained by crossing NAAA conditional knock-in heterozygous mice carrying a NAAA isoform-1 coding sequence within the Rosa26 locus with CD11b-Cre transgenic mice. EAE was induced in NAAA ki mice and wild type littermates, clinical signs became evident 10 days post immunization in both groups, however NAAA ki mice showed significantly higher clinical sign scores than WT littermates. Moreover the abrupt weight loss that accompanies EAE onset was greater in NAAA ki mice than in WT mice.

Our preliminary data suggest that the modulation of NAAA activity might be beneficial for the treatment of MS.

P 68/10

THE SMALL GTP-BINDING PROTEIN RHES INFLUENCES MIDBRAIN DOPAMINERGIC NEURONS: BEHAVIORAL AND BIOCHEMICAL STUDIES

Pinna Annalisa[1], Napolitano Francesco[4], Costa Giulia[2], Wardas Jadwiga[3], Porceddu Pier Francesca[2], Casu Maria Antonietta[5], Contu Liliana[2], Usiello Alessandro[6], Morelli Micaela[2]

[1]National Research Council of Italy (CNR), Neuroscience Institute ~ Cagliari ~ Italy, [2]Dept of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [3]Dept of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences ~ Krakow ~ Poland, [4]CEINGE Biotecnologie Avanzate and Dept. of Molecular Medicine and Medical Biotechnology, Univ. of Naples "Federico II" ~ Napoli ~ Italy, [5]National Research Council of Italy, Institute of Translational Pharmacology, UOS of Cagliari, Scientific and Technological Park of Sardinia POLARIS ~ Pula (CA) ~ Italy, [6]CEINGE Biotecnologie Avanzate and Dept of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples ~ Napoli ~ Italy

Rhes is a small GTP-binding protein highly enriched in the striatum, developmentally regulated by thyroid hormone, and by dopamine (DA) innervation in adult rat brain. Within the striatum, Rhes mRNA is localized in DA D_1 and D_2 receptors-bearing medium-sized projection neurons, as well as in large aspiny cholinergic interneurons, where it modulates DA-dependent transmission and signaling. Interestingly, recent findings showed that Rhes binds and activates mTORC1, a key modulator of several biological processes, including L-DOPA-induced dyskinesia.

This study was aimed to evaluate the effect of Rhes deletion on the number of midbrain dopamine neurons, as well as nigrostriatal-sensitive motor behavior during aging in Rhes mutant mice.

Motor performance and coordination were evaluated using the beam-walking test. Three separate groups of Rhes-/- and Rhes+/+ (control) mice 3, 6 and 12 months old were tested. Time to traverse the beam, number of steps and errors per step were recorded for each animal. Immunohistochemistry and stereological analysis of tyrosine hydroxylase (TH)-positive neurons in SNc were performed in 6 and 12-month-old Rhes+/+ and Rhes -/- mice.

A significant decrease of total number and density of TH-positive neurons was found in 6 and 12 months old Rhes-/- mice as compared with control Rhes+/+ mice of the same age. Three, 6 and 12 months old Rhes-/- mice made significant higher number of steps to traverse the beam compared to Rhes+/+ mice. Six and 12 months old Rhes-/- mice spent significant longer time to traverse the beam compared to Rhes+/+ mice. Moreover, 12 months old Rhes-/- mice made significantly more errors per step in traversing the beam compared to Rhes+/+ mice. Results showed that the motor performance and coordination of Rhes-/-mice is worsened as compared with control mice and it is exacerbated with age.

Overall, these data demonstrated that lack of the Rhes gene in mutant mice results in subtle TH-positive neuron reduction, accompanied by deficits in nigrostriatal-dependent motor behavior.

P 69/10

MOTOR AND COGNITIVE CHARACTERIZATION OF RHES KO FEMALE MICE

Contu Liliana[1], Napolitano Francesco[2], Costa Giulia[1], Porceddu Pier Francesca[1], Wardas Jadwiga[3], Pinna Annalisa[1], Usiello Alessandro[4], Morelli Micaela[1]

[1]Dept. Biomedical Sciences ~ Cagliari ~ Italy, [2]Dept. of Molecular Medicine and Medical Biotechnology ~ Napoli ~ Italy, [3]Dept. of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland ~ Krakow ~ Poland, [4]Dept. of Environmental, Biological and Pharmaceutical Sciences and Technologies, Caserta, Italy ~ Caserta ~ Italy

Rhes, a GTP binding protein belongs to the RAS superfamily. Its expression is predominantly, localized in the caudate-putamen (CPu), and is regulated by the thyroid hormones. Rhes expression is low during embryonic development and in early postnatal phases, becomes higher postnatal and decreases during adulthood. Rhes protein is involved in the regulation of the dopaminergic system, and several studies illustrate a link between Rhes and dopamine-mediated behavior.

To clarify the role of Rhes protein in female mice, we evaluated the presence of motor and cognitive deficits through several behavioral tests in the wild-type Rhes+/+ and knockout (KO) Rhes-/- mice.

A battery of behavioral tests was performed in order to assess: spontaneous motor activity (motility test), motor performance and coordination (beam-walking test and pole test), muscle strength of the forepaw (inverted grid test) and olfactory deficit (buried pellet test). Moreover, we evaluated cognitive ability, using the novel object recognition test and Y maze tests. For this scope, three separate groups of female Rhes-/- and Rhes+/+ (control) mice 3, 6 and 12 months old were tested.

Female Rhes-/- mice, 3, 6 and 12 months old did not showsubstantial differences in motility test, motor performance and coordination, strength of the forepaw, olfactory test and cognitive performance as compared with female Rhes+/+ mice of the same age.

Overall, these data demonstrated that, in contrast to male mice, lack of the Rhes gene in mutant female mice did not produce significant deficits in several motor and cognitive behaviors.

P 70/10

THE MONOMERIC GTP-BINDING PROTEIN RHES INFLUENCES THE NUMBER OF MIDBRAIN DOPAMINERGIC NEURONS AND THE MOTOR DISTURBANCES ASSOCIATED TO L-DOPA IN 6-OHDA-LESIONED MICE

Usiello Alessandro[1], Napolitano Francesco[2]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples (SUN) ~ Caserta ~ Italy, [2]University of Naples "Federico II",Department of Molecular Medicine and Medical Biotechnology ~ Napoli ~ Italy

Rhes is a small GTP-binding protein highly enriched in the striatum, developmentally regulated by thyroid hormone, and by dopamine (DA) innervation in adult rat brain. Within the striatum, Rhes mRNA is localized in DA D_1R- and D_2R-bearing medium-sized projection neurons, as well as in large aspiny cholinergic interneurons, where it modulates DA-dependent transmission and signaling. Previous findings indicate that Rhes, a striatal-enriched small G protein, accounts for the unique neuropathology of Huntington's disease by enhancing mutant huntingtin sumoylation and toxicity. Interestingly, Rhes is also able to bind and activate mTOR.

We first evaluated Rhes mRNA expression pattern in midbrain regions, then we investigated the potential involvement of this GTPase in regulating the number and density of DA neurons and nigrostriatal-sensitive behavior during aging. Finally, we assessed whether Rhes might influence the striatal mTOR-dependent L-DOPA-induced dyskinesia in the hemi-parkinsonian Rhes KO mouse model.

Radioactive in situ hybridization was assessed in adult mice. Beam-walking was executed in 3-,6- and 12-month-old mice. Immunohistochemical staining and stereological analyses of midbrain tyrosine hydroxylase (TH)-positive neurons were performed in 6- and 12-month-old mice. Unilaterally 6-OHDA-lesioned mice were analysed for the anti-akinetic effect of L-DOPA before and 1 h after the first injection of 10 mg/kg L-DOPA in the cylinder test. 6-OHDA-lesioned mice were treated for 9 consecutive days with one injection per day of 10 mg/kg L-DOPA plus benserazide (20 mg/kg). AIMs were assessed at day 3, 6 and 9 by an observer blind to the mouse treatment.

Rhes mRNA is expressed in TH-positive neurons of substantia nigra and ventral tegmental area. Moreover, lack of Rhes leads to roughly 20% of nigral TH-positive neuronal loss in both 6- and 12-month-old mutants, when compared to their age-matched controls. Rhes mutants display subtle alterations in motor coordination, as measured by beam-walking test. Moreover, 6-OHDA-lesioned Rhes KO mice show a significant reduction of L-DOPA-induced dyskinesia, via enhancement of the striatal mTOR signaling, without affecting the therapeutic improvement of forelimb movement.

Our findings indicate a subtle although significant role of Rhes in regulating the number of TH-positive neurons of substantia nigra and nigrostriatal–sensitive behavior. In addition, given the negligible levels in peripheral tissues, drugs blocking Rhe-mTOR interactions may have much less potential for adverse effects, consistent with the lack of major abnormalities in mutant mice.

P 71/10

ENDOCANNABINOIDS LEVELS IN SPECIFIC CEREBRAL AREAS OF RAT FOLLOWING NITROGLYCERIN ADMINISTRATION: A POSSIBLE ROLE IN MIGRAINE PAIN

Demartini Chiara[1], Greco Rosaria[2], Bandiera Tiziano[3], Guijarro Ana[3], Mangione Antonina Stefania[2], Armirotti Andrea[3], Nappi Giuseppe[2], Blandini Fabio[4], Sandrini Giorgio[1], Piomelli Daniele[3], Tassorelli Cristina[1]

[1]Laboratory of Neurophysiology of Integrative Autonomic Systems, Headache Science Centre, "C. Mondino" National Neurological Institute; Department of Brain and Behavioural Sciences, University of Pavia ~ Pavia ~ Italy, [2]Laboratory of Neurophysiology of Integrative Autonomic Systems, Headache Science Centre, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy, [3]Medicinal Chemistry Drug Discovery and Development, Italian Institute of Technology ~ Genova ~ Italy, [4]Laboratory of Functional Neurochemistry, Center for Research in Neurodegenerative Diseases, "C. Mondino" National Neurological Institute ~ Pavia ~ Italy

Systemic nitroglycerin (NTG) induces in rat a state of trigeminal and spinal hyperalgesia through the neuronal activation of brain areas involved in migraine pain. Experimental evidence shows the antinociceptive effect of endocannabinoids and their role in the modulation of trigeminovascular system activation, suggesting that the endocannabinoid system may be dysfunctional in migraine. In a previous study, we have reported significant changes in the activity of enzymes that catabolize anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in the brainstem and hypothalamus of rats following NTG administration. In the same areas, we also observed an up-regulation of CB1 receptors binding sites.

To evaluate NTG-induced changes of endocannabinoids levels in peripheral and central areas implicated in migraine pain.

In this study male Sprague-Dawley rats were injected i.p. with NTG or vehicle and sacrificed 4 hours later. The meninges, mesencephalon, medulla and cervical spinal cord were dissected out and utilized for the evaluation of the endocannabinoids and endocannabinoid-related molecules by liquid chromatography-mass spectrometry. More specifically, we quantified the levels of AEA, 2-AG, palmitoylethanolamide (PEA) and N-oleoyl ethanolamine (OEA).

Systemic NTG induced a significant decrease in 2-AG levels in the meninges and a significant increase in 2-AG levels in the mesencenphalon. No significant differences in cerebral AEA levels were observed between NTG and vehicle groups, although a tendency toward an increase was observed in the meninges after NTG administration. A tendency toward a decrease in PEA levels was found in meninges of NTG group. No significant changes were observed in the meningeal and brain levels of OEA.

Systemic NTG administration induces a significant and specific reduction in 2-AG levels in the meninges and in the mesecenphalon of rats. Combined with previous data from our group, this finding suggests a key role of 2-AG in migraine pain.

P 72/10

HEART RATE VARIABILITY (HRV) AND CARDIOVASCULAR MODULATION IN PARKINSON'S DISEASE PATIENTS WITH TREMOR DOMINANT AND AKINETIC RIGID DOMINANT SUBTYPES

Solla Paolo[1], Cadeddu Christian[2], Cannas Antonino[1], Deidda Martino[2], Mura Nicola[2], Mercuro Giuseppe[2], Marrosu Francesco[1]

[1]Department of Public Health, Clinical and Molecular Medicine, Movement Disorders Centre, University of Cagliari, Cagliari, Italy ~ Cagliari ~ Italy, [2]Department of Medical Sciences ''Mario Aresu'', University Hospital of Cagliari, University of Cagliari, Strada Statale 554, Km 4.500, Monserrato, 09042 Cagliari, Italy ~

Parkinson's disease (PD) patients can be differentiated in separate motor subtypes depending on the predominant symptoms (tremor or rigidity/bradykinesia). Different degrees of cognitive decline and slower disease progression are often observed in tremor dominanant (TD) patients compared to those with akinetic-rigid dominant (ARD) subtype. Cardiovascular dysautonomia is often observed in PD patients, although the definite correlation with different subtypes of PD is not clear. In this scenario, heart rate variability (HRV) analysis represents a non-invasive and established tool in assessing cardiovascular autonomic modulation.

To investigate the cardiovascular autonomic modulation in PD patients with TD subtype in comparison to ARD subtype subjects using HRV analysis.

Twenty-eight PD patients (17 with TD subtype and 11 with ARD subtype) were enrolled and compared to 17 age and sex-matched healthy controls. HRV was analyzed in time- and frequency-domains.

Low-frequency (LF) values were significantly lower in the ARD subtype with respect to TD group [LF 41.4 ± 13.6 vs 55.5 ± 11.6 ($p<0.007$)] indicating a more evident impairment of the baroreflex modulation of the autonomic outflow mediated by both sympathetic and parasympathetic systems in the first class of patients.

Our observations support the relevance of difference between TD and ARD subtypes, supporting the idea of a different pathophysiological process between these forms. These differences also support the idea that different subtypes may also result in different responses to therapy or in the possible development of cardiovascular side effects of dopaminergic drugs in these different groups of patients.

P 73/10

ENDOCANNABINOID SYSTEM: POSSIBLE NEW TARGET IN THE TREATMENT OF ANOREXIA NERVOSA

Collu Roberto[1], Scherma Maria[1], Satta Valentina[1], Bratzu Jessica[1], Castelli Maria Paola[1], Boi Maria Francesca[2], Usai Paolo[2], Fattore Liana[3], Fadda Paola[1], Fratta Walter[1]

[1]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari ~ Cagliari ~ Italy, [2]Department of Internal Medicine, University of Cagliari ~ Cagliari ~ Italy, [3]CNR Neuroscience Institute Cagliari, National Research Concil ~ Cagliari ~ Italy

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-V Anorexia Nervosa (AN) is considered a chronic and disabling psychiatric pathology characterized by a disturbed body image perception and aberrant eating patterns associated with weight-control behaviors, such as excessive dieting and extreme physical exercise (APA, 2013; Shroff et al., 2006). It is well known that the endocannabinoid system (ECs) plays a modulatory role in both the hedonic and homeostatic aspects of eating behavior and for this reason its dysregulation could play a role in the pathophysiology of AN (Di Marzo and Matias, 2005). In fact, plasma concentrations of the endogenous cannabinoid anandamide were found significantly enhanced in patients with AN (Monteleone et al., 2005). Moreover, a recent brain imaging study showed an altered density of cannabinoid CB1 receptors (CB1r) in cortical and subcortical brain areas (Gèrard et al, 2011). In addiction, brain imaging studies have shown both neuroanatomical abnormalities and dysfunctional activation of brain areas modulating reward in AN patients (Kaye et al., 2009; Keating et al., 2012; Holsen et al., 2012).

On the basis of these findings, the goal of our study was to elucidate the role of the ECs and its possible neuroanatomical alterations in the pathophysiology of AN and to investigate whether its pharmacological modulation could be able to modify symptomatology in a widely validated model of AN.

In the “activity-based anorexia” (ABA) paradigm, the combination between a rigid dietary restriction and the availability of running wheels create a simil-anorexic condition in which animals dramatically lose body weight and progressively increase running wheel activity (RWA).

Our results demonstrate that restricted-feeding schedule selectively altered CB1r density in feeding-related brain areas such as prefrontal cortex and hypothalamus in ABA animals. We have seen that pharmacological treatment with both natural CB_1/CB_2 (Δ^9-tetrahydrocannabinol) and synthetic CB_1 (CP55,940) receptor agonists attenuated weight loss and RWA, while, treatment with the CB_1r inverse agonist/antagonist rimonabant did not modify either body weight or RWA. Moreover, our data revealed that plasma levels of leptin were decreased in ABA animals, while, ghrelin and corticosterone levels were increased. Changes in these plasma levels were found in ABA animals after pharmacological treatments with both agonists and antagonists tested.

Taken together our results demonstrate the involvement of the ECs in the pathophysiology of AN and suggest that pharmacological therapies based on its modulation might be effective in the treatment of this eating disorder.

P 74/10

ALS MODELS, A CONTRIBUTE FROM PATIENT'S LYMPHOBLASTOID CELL CULTURE

Pansarasa Orietta[1], Bordoni Matteo[2], La Salvia Sabrina[3], Valente Marialuisa[1], Drufuca Lorenzo[1], Diamanti Luca[4], Gagliardi Stella[1], Cereda Cristina[1]

[1]Laboratory of Experimental Neurobiology, C. Mondino National Institute of Neurology Foundation, IRCCS, ~ Pavia ~ Italy, [2]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia ~ Pavia ~ Italy, [3]Department of Public Health, Neuroscience, Experimental and Forensic Medicine, University of Pavia, ~ Pavia ~ Italy, [4]Department of Brain and Behavioural Science, University of Pavia ~ Pavia ~ Italy

In ALS, it is difficult to identify a valid pathological model. MNs would be the best candidates, unfortunately they are available only post-mortem and give little information on early disease stages. Cellular and animal models are useful, however translation to patients is laborious and not completely reliable. Regarding patients' peripheral tissues, PBMCs from sALS patients display signs of oxidative stress reflecting MNs modifications (Cereda et al, 2013; Cova et al, 2006).

We verify if the molecular signatures could be associated with gene mutation (SOD1, TARDBP, FUS) in PBMCs from ALS patients. Because PBMCs cannot be grown and stored as a cell line and do not show the handling flexibility needed for "modeling", we switched our attention to lymphoblasts, to verify if PBMCs molecular signatures were maintained.

SOD1, TARDBP and FUS transcript levels were analyzed in PBMCs from mutated, sALS patients and controls. SOD1 mRNA was evaluated in lymphoblasts from SOD1mut patients to verify PBMCs result reproducibility. In lymphoblasts, we investigate SOD1, TDP43 and FUS cellular localization by WB and immunofluorescence. Apoptosis and mitochondrial dynamics were analyzed by WB. Mitochondrial morphology was studied by TEM.

PBMCs from SOD1mut patients showed SOD1 mRNA altered levels, the mutated allele was up-regulated. In lymphoblasts SOD1 mRNA levels increased. In total soluble fraction, SOD1 protein levels were reduced and immunofluorescence showed SOD1 recruitment in cytoplasmic aggregates. No changes in Cyt-C release were observed; a slight increase in DRP1 levels indicated that fission is favored. TEM evidenced smaller mitochondria with disorganized cristae and vacuoles. TARDBPmut patients showed increased levels of TARDBP transcripts. Lymphoblasts cytoplasmic extracts presented high levels of the protein. Immunofluorescence showed cytoplasm round-shaped TDP43-containing aggregates. An increased cytoplasmic Cyt-C release suggested apoptosis and elevated MFN1 levels promoted the fusion pathway. Mitochondria presented giant masses containing electrondense globes and vacuoles. In FUSmut patients, mRNA levels did not change and no protein mislocalization was observed. Cytoplasm Cyt-C release suggested apoptosis. MFN1 levels were increased and TEM reported giant and degenerated mitochondria.

Patient-derived lymphoblasts display features typical of degenerating MNs: impaired RNA metabolism, protein aggregation and mitochondrial dysfunction. Lymphoblasts are intriguing as ALS cellular model to study specific pathological pathways or identify new ones. Since patients'

blood is relatively easy to obtain and lymphoblastoid culture can simply be settled, it is possible to collect mutation-specific samples, allowing a comparison between pathological signatures of different mutations, leading to patient stratification on a molecular basis.

P 75/10

CHARACTERIZATION OF THE INTERACTION BETWEEN ALPHA-SYNUCLEIN AND PRION PROTEIN: A PUTATIVE OVERLAP OF TWO NEURODEGENERATIVE DISEASES

Legname Giuseppe

SISSA ~ Trieste ~ Italy

Several studies report the involvement of the cellular prion protein (PrPC) in binding and modulating the toxicity of proteins involved in neurodegenerative disorders (such as Aβ oligomers in Alzheimer's disease). In this work we investigated whether this is also true for α-synuclein (α-syn), a protein involved in neurodegeneration in Parkinson's disease (PD).

To test whether α-syn and PrPC do interact during the propagation of aggregates in neurodegenerative diseases.

Using the same methodology adopted to obtain synthetic mammalian prions, we formed recombinant mouse α-syn amyloids. Subsequently, we characterized various preparations of α-syn amyloids and explored the uptake of these preparations in neuroblastoma N2a cells which express PrPC (N2aWT) and N2a cells knocked out for PrPC protein (N2aKO).

Our results show that the uptake of α-syn amyloids is different in N2aKO if compared to control cells. Confocal microscopy and co-localization with sub-compartmental markers revealed that the α-syn amyloids co-internalized with PrPC, accumulated and trafficked to lysosomes. Furthermore, serial passages of N2aWT cells treated with α-syn amyloids led to sustained accumulation of both, α-syn and PrP. Further work was required to validate the importance of this interaction in disease progression in vivo. Thus, we performed stereotaxic injections in substantia nigra pars compacta of α-syn amyloids in FVB PrPWT and FVB PrPKO mice.

Our findings suggest a role for PrPC in regulating of α-syn aggregates uptake, thus, evidencing a link between the two neurodegeneration associated proteins. This study suggests an overlap between prion disease and PD.

P 76/10

TRACTOGRAPHY OF WHITE MATTER CONNECTIONS PREDICTS FOR VASCULAR COGNITIVE IMPAIRMENT IN HYPERTENSIVE PATIENTS

Carnevale Lorenzo[1], Selvetella Giulio[1], Cugino Daniela[1], Grillea Giovanni[1], Lembo Giuseppe[2], Carnevale Daniela[2]

[1]IRCCS Neuromed ~ Pozzilli ~ Italy, [2]Sapienza University of Rome and IRCCS Neuromed ~ Pozzilli ~ Italy

Vascular cognitive impairment (VCI) results by several vascular risk factors and, particularly, hypertension (HTN). The identification of early changes associated with later development of dementia is demanding. Great part of research has primarily focused on brain changes occuring in grey matter. However, more recent data highlighted that HTN may determine cognitive decline, even before manifest neurodegeneration. Diffusion tensor imaging (DTI) on magnetic resonance, opened the possibility to predict white matter connections that correlate with specific cognitive functions.

In this study, we used DTI and cognitive assessment (CA), in order to identify a regional pattern of fractional anisotropy (FA) changes that could predict for VCI in hypertensive patients (HT).

We have examined 15 HT (moderate to severe, with antihypertensive medications) vs 15 normotensive (NT), subjecting them to DTI and CA. Clinical data were recorded as well.

HT had significant higher SBP (138 ± 4 vs 118 ± 3 in NT) and DBP (87 ± 2 vs 75 ± 2 in NT) ($p<0.001$), displayed a significant LV hypertrophic remodeling (LVM/BSA 112 ± 5 vs 83 ± 3 for NT) ($p<0.0001$), with a significant moderate increase in albuminuria (15.7 ± 2.6 mg/24 h vs 8.8 ± 1.6 for NT) ($p<0.03$). When subjected to CA, HT had significantly worse performance on both MoCA (22.66 ± 0.97 vs 26.21 ± 0.57 NT) and Stroop Test (34.50 ± 3.87 vs 17.75 ± 2.57 NT) ($p<0.01$). Conversely, tests regarding Verbal Fluency and Instrumental Activities of Daily Living revealed normal performance of HT, thus indicating a selective impairment of memory. Brain imaging showed that, while none of the patients had abnormal signal intensity on T1/T2weighted MRI, DTI indices FA were significantly reduced in HT as vs NT. In particular, HT had lower FA in projection fibers related to impairment for nonverbal materials (Anterior Thalamic Radiation: 0.358 ± 0.012 vs 0.330 ± 0.006, $p<0.05$), association fibers involved in executive functioning and emotional regulation (Superior Longitudinal Fasciculus: 0.388 ± 0.013 vs 0.356 ± 0.007, $p<0.05$), limbic system fibers involved in attention tasks (cingulate gyrus: 0.364 ± 0.009 vs 0.328 ± 0.010, $p<0.01$).

Our data highlight a novel paradigm of combined DTI/CA of HT patients, capable to identify, with great sensitivity, predictive signs of HTN-induced VCI.

P 77/10

EFFECT OF SIGMA-1 RECEPTOR (S1R) AGONISTS ON THE DISEASE PROGRESSION OF SOD1.G93A MICE: LIGHTS AND SHADOWS.

Secomandi Nicola[1], Vallarola Antonio[2], Peviani Marco[1], Sarno Nicole[1], Petese Alessandro[1], Marra Annamaria[3], Rossi Daniela[3], Collina Simona[3], Curti Daniela[1]

[1]Dept. Biology and Biotechnology, University of Pavia ~ Pavia ~ Italy, [2]Dept. Neuroscience, IRCCS Mario Negri ~ Milan ~ Italy, [3]Dept. Drug Sciences, University of Pavia ~ Pavia ~ Italy

Amyotrophic lateral sclerosis (ALS) is a multifactorial motoneuron (MN) disease, with a complex interplay between genetic and epigenetic factors. Riluzole, the only drug approved for ALS treatment, has limited efficacy and poorly understood mechanism of action. S1R is an orphan receptor with chaperone-like activity, enriched in spinal cord MNs and mainly localized at the mitochondria-associated-ER membranes (MAMs). Mutations in S1R have been found in familial cases of ALS. S1R knockout causes locomotor impairment, axonal degeneration, MN loss, and exacerbates disease progression in SOD1.G93A mice. S1R agonists are neuroprotective both in vitro and in vivo. Thus, S1R has emerged as a promising therapeutic target to improve MN survival in ALS.

To test the effect of treatments with the well known S1R agonist PRE-084 and with a novel S1R agonist (NS1R, synthesized by the MEDCHEM lab, at the UniPV), on the disease progression of SOD1.G93A mice.

SOD1.G93A mice were administered PRE-084 (0.25 mg/kg die i.p.) or NS1R (0.1 mg/kg, die i.p.) for 5 weeks or until survival, starting from the 14th week of age (presymptomatic stage). Body weight, extension reflex, rota-rod and grip strength performance were assessed twice a week at the AriSLA Facility (IRCCS, M.Negri). MN survival and glial reactivity were measured by immunocytochemistry at the 19th week of age and at the disease end stage. S1R signal was assessed with anti-S1R antibody and DAB Peroxidase (HPR) staining. Spinal cord levels of the S1R agonists were measured (HPLC-MS) 24 h after the last administration. Two-way ANOVA and Tukey test or Kaplan-Meier and Log-rank test were used for statistical analyses.

The major benefits of the treatment were observed after 5 weeks when extension reflex and rota-rod performance were significantly improved. MN survival was increased ($p<0.01$), astrocytosis and microgliosis were reduced ($p<0.001$) compared with vehicle treated mice. At the end stage, neither animal survival (mean ± SD days, vehicles: 162 ± 8; PRE-084: 171 ± 10; NS1R: 171±12) nor the neurochemical parameters were significantly ameliorated by the S1R agonists at the dosage utilized in this study. Both PRE-084 and NS1R were not detectable in the spinal cord of SOD1.G93A mice at the end stage of the disease.

S1R agonists display potential benefits for treating ALS even if the drug dosage protocol we have used in this study is suboptimal. The efficacy of the S1R agonists decreases with disease progression possibly because of difficulty to reach their target (modified pharmacokinetics and/or pharmacoresistance).

P 78/10

INTENSIVE REHABILITATION ENHANCES LYMPHOCYTE BDNF-TRKB SIGNALING IN PATIENTS WITH PARKINSON'S DISEASE

Frazzitta Giuseppe[2], Fontanesi Cecilia[1], Kvint Svetlana[1], Ferrazzoli Davide[2], Bera Rossana[2], Di Rocco Alessandro[3], Pezzoli Gianni[4], Quartarone Angelo[5], Wang Hoau Yan[1], Ghilardi Maria Felice[1]

[1]Department of Physiology, Pharmacology & Neuroscience, CUNY Medical School, ~ New York, NY, USA, 10031 ~ United States, [2]Department of Parkinson Disease and Brain Injury Rehabilitation, "Moriggia Pelascini" Hospital, ~ Gravedona ed Uniti ~ Italy, [3]Department of Neurology, Movement Disorders Center, NYU-Langone School of Medicine, ~ New York, NY, 10016 ~ United States, [4]Parkinson Institute - Istituti Clinici di Perfezionamento ~ Milano ~ Italy, [5]Department of Neurosciences, University of Messina, ~ Messina ~ Italy

In a combined animal and human study, we have previously found that a five-day treatment that enhances cortical plasticity also facilitates brain-derived neurotrophic factor (BDNF)-tyrosine receptor kinase B (TrkB) signaling and increases activated TrkB and N-methyl-D-aspartate receptor (NMDAR) association in both the cortex and the peripheral lymphocytes. Patients with Parkinson's disease (PD) in general show decreased cortical plasticity, as demonstrated by electrophysiological and behavioral studies.

Here we test the hypothesis that an exercise program that improves motor function and seems to slow down symptoms' progression can enhance BDNF-TrkB signaling in lymphocytes.

Sixteen patients with PD underwent a four-week Multidisciplinary Intensive Rehabilitation Treatment (MIRT), which included aerobic training, physical and occupational therapy. Blood was collected before, after two- and four-week MIRT. Lymphocytes were isolated to examine BDNF-TrkB signaling induced by incubation with recombinant human BDNF. TrkB signaling complexes, extracellular-signal-regulated kinase-2 and protein-kinase-B were immunoprecipitated; content of immunocomplexes was determined by Western blotting.

After MIRT, all patients showed improvement in motor function. TrkB interaction with NMDAR and BDNF-TrkB signaling increased in peripheral lymphocytes at receptor, intracellular mediators and downstream levels.

We conclude that MIRT promotes changes of the immune function in PD. The reduced severity of PD symptoms together with enhanced lymphocyte BDNF-TrkB signaling further suggests that the immune system might play a role in neurorestoration and recovery of function. We finally speculate that, as BDNF-TrkB signaling in cortex and lymphocyte are partially correlated, MIRT might also increase cortical plasticity.

P 79/10

PHARMACOLOGICAL CHARACTERIZATION OF THE SYNTHETIC CANNABIMIMETIC COMPONENTS OF "SPICE" DRUGS: FOCUS ON ABUSE LIABILITY

Loi Barbara[2], De Luca Maria Antonietta[1], Miliano Cristina[1], Porcu Alessandra[1], Martorelli Mariella[1], Davidson Colin[3], Castelli Maria Paola[1], Schifano Fabrizio[2], Di Chiara Gaetano[1]

[1]Department of Biomedical Sciences, University of Cagliari ~ Cagliari ~ Italy, [2]School of Life and Medical Sciences, University of Hertfordshire ~ Hertfordshire ~ United Kingdom, [3]Pharmacology and Cell Physiology, Division of Biomedical Science, St George's University of London ~ London ~ United Kingdom

Spice is a smokable herbal mixture marketed as a legal alternative to Cannabis. Spice is composed by shredded plant material laced with a variety of synthetic cannabimimetic compounds. New legal regulations have been enacted to control the Spice global diffusion. As a consequence of that, three subsequent generations of synthetic cannabimimetics have been developed based around slight modifications of the first generation compound JWH-018. Among the recently emerged third generation compounds, STS-135, 5F-AKB48, 5F-PB-22 and BB-22 have been detected in several specimens of Spice. We previously characterized the neurobiological mechanism underlying JWH-018 rewarding properties. According to these studies, JWH-018 was found to be self-administered by rodents, able to stimulate dopamine (DA) transmission preferentially in the nucleus accumbens (NAc) shell as compared to NAc core and medial-prefrontal cortex (mPFC), and effective in decreasing GABAA-mediated post-synaptic currents in VTA DA neurons.

In order to evaluate the abuse liability of the third generation Spice drugs, we compared the pharmacological properties of STS-135, 5F-AKB48, 5F-PB-22 and BB-22 with those previously obtained in JWH-018 studies.

By in vitro [3H]CP55,940 and GTPgS binding performed in rat cortex homogenate, we evaluated the affinity and the intrinsic activity of these compounds at the CB_1 receptor. GTPgS binding assay was also performed in the presence of either the CB_1 receptor antagonist/inverse agonist AM251 (0.1 μM) or the neutral antagonist O2050 (1 μM). By in vivo microdialysis studies in rats, we evaluated the effect of BB-22 (0.003-0.1 mg/kg iv) on DA transmission in the NAc shell and core and mPFC.

In vitro studies showed that JWH-018 inhibited [3H]CP55,940 binding with a Ki of ~ 3.39 nM, all the other compounds showed a statistically significant lower Ki as follows: STS-135 ~ 1.9 nM; 5FAKB48 ~ 0.87 nM; 5F-PB-22 and BB-22 ~ 0.09 nM. Moreover, all compounds stimulated GTPgS in a concentration-dependent manner (EC50: STS-135, 32.7 nM; 5F-AKB48, 30 nM; BB-22, 3.7 nM; 5F-PB-22, 2.9 nM). EC50s of BB-22 and 5F-PB-22 were significantly lower than JWH-018 (20 nM). In vivo studies showed that BB-22, at the dose of 0.01 mg/kg iv, increased DA release in the NAc shell but not in the NAc core and mPFC. Lower (0.003 mg/kg) and higher doses (0.03 and 0.1 mg/kg) were ineffective.

These findings link these components of Spice to other cannabimimetics with recognised abuse potential, and to other classes of abused drugs that increase DA signal in the NAc shell.

P 80/10

EPIGENETIC AND TRANSCRIPTIONAL DYSFUNCTION IN ISCHEMIC BRAIN INJURY AND HUNTINGTON'S DISEASE: FROM MECHANISMS TO THERAPEUTIC CANDIDATES

Yildirim Ferah[1], Ng Christopher W , Gipson Theresa A, Vashishtha Malini, Harms Christoph, Meisel Andreas, Thompson Leslie M., Housman David E., Fraenkel Ernest

[1]Department of Psychiatry and Human Behavior and UCI Institute of Memory Impairments and Neurological Disorders.

Aberrant changes in gene expression patterns along with impaired epigenetic regulation play pivotal roles in the pathogenesis of central nervous system diseases. In line, strategies that target epigenetic and gene expression dysfunction such as histone deacetylase (HDAC) inhibition lead to robust beneficial effects in numerous neurological and psychiatric conditions.

Our goal is to evaluate the epigenetic and gene expression changes in neurons in disease to identify the key pathogenic changes in regulation of gene expression. These processes are to become candidates for novel therapeutic interventions, potentially for a range of neurological and psychiatric diseases.

We use a combination of genome-wide DNA sequencing technologies, e.g. chromatin immunoprecipitation (ChIP-Seq) to assess the status of histone modifications and RNA sequencing (RNA-Seq) to measure transcription throughout the genome, together with molecular biology techniques and appropriate cell and mouse models of disease in our studies.

We observed impaired neuronal histone acetylation levels in brain ischemia models and identified cAMP-response element binding protein (CREB)–binding protein (CBP) as a crucial factor in the susceptibility of neurons to ischemic stress. In accordance with our previous results that show neuroprotection by histone acetylation enhancement induced by HDAC inhibition, in the present study, ischemic preconditioning increased histone acetylation levels globally as well as at specific neuroprotective gene promoters extending the role of epigenetic regulation also to endogenous neuroprotection programs. In Huntington's disease (HD), genome-wide analysis of transcription and histone H3K4me3, an active epigenetic mark, revealed highly coordinated chromatin changes along with the detected transcriptional failure in the affected brain areas in R6/2 mice. Targeting the levels of a H3K4-specific histone demethylase in in vitro HD models rescued down-regulation of key neuronal genes caused by mutant Huntingtin expression and proved protective in a Drosophila HD model.

Our work on brain ischemia suggests that histone acetylation and its machinery, e.g. CBP, determine stroke outcome and play crucial roles for the induction of ischemia-resistance in neurons. Our Huntington's disease studies identified H3K4me3 as a novel therapeutic target for ameliorating the disease progression in HD. Altogether, our findings show that the epigenome and transcription change in coordination in disease and the epigenetic processes provide unprecedented therapeutic opportunities for rescuing the transcriptional demise thereby achieving protection in ischemic brain injury, Huntington's disease and potentially in other central nervous system disorders.