



IN – INSTITUTE OF NEUROSCIENCE
Annual retreat – 2016
Padova, Sept. 13-16, 2016
**ORAL COMMUNICATIONS: SCHEDULE &
 ABSTRACTS**



N	When	Speaker	Title
Invited Lectures			
IL1	T 16:10	Vincenzo Di Marzo	<i>The endocannabinoid system and its interactions with orexinergic signaling in food intake, pain perception and neuroprotection</i>
IL2	T 17:00	Attilio Iemolo	<i>Role of central neuropeptides in regulating stress and eating behaviors</i>
IL3	W 10:00	Laura Cancedda	<i>Potentials for Rescuing Cognitive Deficits in Neurodevelopmental Disorders by early intervention: the Case of Down Syndrome.</i>
IL4	W 12:20	Alessandro Sale	<i>The Brain-Boosting Power of Environmental Enrichment</i>
IL5	W 17:50	Mathew Diamond	<i>Using time to generate a touch percept and touch to generate a time percept</i>
IL6	W 21:00	Alessandro Angrilli	<i>Altered hemispheric asymmetry of language in psychoses: testing an evolutionary theory</i>
IL7	Th 11:30	Francesco Tombola	<i>The Yin and Yan of mechanosensing: the tale of two channels</i>
Oral communications			
OC session 1. Physiology and biochemistry of behaviour and reward			
OC1.1	T 14:30	Cristina Cadoni	<i>Adolescence vs. adulthood: differences in basal dopamine transmission and response to drugs of abuse</i>
OC1.2	T 14:50	Giancarlo Colombo	<i>Another chapter of the “drinking in the dark” saga: exacerbation of anxiety underlying the stronger urge for alcohol late at night</i>
OC1.3	T 15:10	Claudia Sgheddu	<i>Elevation of endogenous kynurenic acid levels suppresses Δ^9-tetrahydrocannabinol-induced effects on the mesocorticolimbic circuit</i>
OC1.4	T 15:30	Linda Maria Requeie	<i>Endocannabinoid signaling to astrocytes in the ventral tegmental area and modulation of synaptic transmission</i>
OC1.5	T 15:50	Bice Chini	<i>KCC2 is a key target of oxytocin in postnatal events potentially involved in the pathogenesis of neurodevelopmental disorders.</i>
OC session 2. Neurological diseases			
OC2.1	W 8:40	Yuri Bozzi	<i>Interneuron defects during postnatal development of somatosensory cortex of engrailed 2 knockout mice</i>
OC2.2	W 9:00	Elisa Focchi	<i>Prenatal exposure to poly I:C increases susceptibility to epilepsy in the adult offspring</i>
OC2.3	W 9:20	Cristina Spalletti	<i>Combining Robotic Rehabilitation with silencing of the healthy hemisphere promotes True Motor Recovery in a Mouse model of Ischemic Injury</i>
OC2.4	W 9:40	Stefano Morara	<i>Calcitonin gene-related peptide (CGRP) acts as an homeostatic factor on microglial cells in experimental autoimmune encephalomyelitis</i>

OC session 3. Neurodegeneration, cell death			
OC3.1	W 11:20	Viviana Guadagni	<i>Targeting retinal inflammation to delay photoreceptor degeneration in Retinitis Pigmentosa</i>
OC3.2	W 11:40	Marco Pirazzini	<i>Regeneration of the paralyzed neuromuscular junction</i>
OC3.3	W 12:00	Angelo Iannielli	<i>iPSC-based in vitro model of Parkinson's disease caused by OPA1 mutations</i>
OC session 4. Communications a la carte			
OC4.1	Th 8:40	Edoardo Moretto	<i>TSPAN5 is a key player in dendritic spine formation and AMPA receptor recycling</i>
OC4.2	Th 9:00	Martina La Spina	<i>Autophagy induction by the natural compound pterostilbene: mechanisms and therapeutic potential</i>
OC4.3	Th 9:20	Arianna Crespi	<i>The cell surface trafficking of the $\alpha 3\beta 4$ neuronal nicotinic acetylcholine receptor is regulated by its accessory subunit</i>
OC4.4	Th 9:40	Ilaria Prada	<i>Glia-to-neuron shuttling of mir-146a via extracellular microvesicles modulates protein expression in neurons</i>
OC4.5	Th 10:30	Barbara Corso	<i>Type I diabetes microvascular complications and interaction with HLA: a linkage analysis</i>
OC4.6	Th 10:50	Nina Kaludercic	<i>Monoamine oxidases cause mitochondrial and endoplasmic reticulum stress leading to cardiomyopathy in type I diabetes</i>
OC4.7	Th 11:10	Manuela Antoniel	<i>The unique histidine of F-ATP synthase subunit OSCP mediates regulation of the permeability transition by matrix pH</i>

THE ENDOCANNABINOID SYSTEM AND ITS INTERACTIONS WITH OREXINERGIC SIGNALING IN FOOD INTAKE, PAIN PERCEPTION AND NEUROPROTECTION

Vincenzo Di Marzo

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The endocannabinoid (eCB) system was originally composed of two G protein-coupled receptors (GPCRs), the cannabinoid CB1 and CB2 receptors, the two main endogenous lipid ligands of such receptors [also known as endocannabinoids (eCBs)], anandamide and 2-arachidonoyl-glycerol (2-AG), and the enzymes responsible for ligand biosynthesis and inactivation. The eCB system is a pleiotropic signalling system involved in all aspects of mammalian physiology and pathology, and hence it represents a potential target for the design and development of new therapeutic drugs. However, the eCBs as well as some of their congeners also interact with a much wider range of receptors, including members of the Transient Receptor Potential (TRP) channels, Peroxisome Proliferator-Activated Receptors (PPARs), and other GPCRs. Indeed, following the discovery of the eCBs, eCB-related lipid mediators, which often share metabolic pathways with the eCBs, have also been identified or rediscovered.

The eCBs and related mediators, also due to their biosynthetic phospholipid origin and lipophilic nature, can only act as local (autocrine or paracrine) mediators and neuromodulators. The anatomical distribution of some eCB metabolic enzymes and molecular targets (for example the CB1 receptor and the TRPV1 channel), makes of these mediators ideal retrograde (as in the case of 2-AG) or anterograde (as in the case of anandamide) modulators of neurotransmitter release and action, and hence of synaptic plasticity, but also of neuropeptide biosynthesis. In the case of the wake and food intake inducer as well as analgesic neuropeptide, orexin-A, recent data from my laboratory showed that eCB signalling can be up-stream, down-stream and synergistic with orexinergic signalling, with potential impact on metabolic disorders, pain perception and tauopathies. These data will be briefly reviewed during my lecture.

ROLE OF CENTRAL NEUROPEPTIDES IN REGULATING STRESS AND EATING BEHAVIORS

Attilio Iemolo

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Obesity is nowadays a serious health problem of worldwide interest. In 2014 more than 2 billion people were either overweight or obese. Obesity is not only a problem of increased body mass index (BMI), but it can also represent a risk factor for other diseases, such as cancer, type-2 diabetes, cardiovascular, mood-related and neurodegenerative diseases. Along with genetic causes, overconsumption of highly palatable food, especially of those rich in sugars and fats, is considered a major factor contributing to the recent surge in obesity. Indeed, palatability of sugars and fats is a key factor in strongly motivating and increasing food consumption even in the absence of an energetic requirement.

Stress experiences are of crucial importance in regulating eating behaviors. Acute stress, stimulating the hypothalamic-pituitary-adrenal (HPA) axis, activates adaptive responses, including suppression of appetite and food intake, to regain equilibrium lost by the impact of the stressor. In this scenario, the hypothalamus, and a number of hypothalamic neuropeptides, such as CRF, pituitary adenylate cyclase-activating polypeptide (PACAP), neuropeptide Y (NPY), agouti-related peptide and proopiomelanocortin (POMC) play a critical role in regulating feeding and energy balance.

However, chronic, intermittent stressors, by altering extrahypothalamic brain regions involved in stress/motivation circuits, could lead to changes in allostatic load where negative reinforcement mechanisms, by dampening stress-related adaptive responses, may drive increasingly compulsive overconsumption of highly palatable food, that eventually promotes weight gain and body fat mass. Given the rewarding properties of foods and drinks high in fat and/or sugar, it is hypothesized that highly palatable food may serve as “comfort food” that acts as a form of self-medication to dissipate unwanted distress.

Understanding the associations and interactions between stress, neurobiological adaptations, and eating behaviors is important in the development of effective prevention and treatment strategies for obesity, metabolic diseases and other unhealthy eating behaviors.

Potentials for Rescuing Cognitive Deficits in Neurodevelopmental Disorders by early intervention: the Case of Down Syndrome

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Neurodevelopmental disorders (ND) are chronic psychiatric conditions with different etiologies, but most share a strong genetic component, defective brain development, and cognitive impairment. Currently, treatment options are very limited, and early educational intervention is the cornerstone for the management of cognitive impairment in most ND, indicating the positive effect of early actions during brain development. Among ND, Down syndrome (DS) is caused by the presence of an extra chromosome 21, and it represents the leading cause of genetically-defined intellectual disability. Different pharmacological treatments targeting one of the many pathways downstream of the triplicated genes have been shown to rescue cognitive impairment in DS animal models. Nevertheless, most of these preclinical studies have been performed postnatally and often in adults, possibly because of concerns of unwanted drug side effects that may have long-lasting noxious sequelae on a developing brain at embryonic stages. On the other hand, viral (but also non-viral) gene therapy approaches in animal models of ND have been mostly neglected because of technical and ethical issues, when considered in the light of future translational applications. Yet, DS is mostly diagnosed prenatally, when many of its brain developmental abnormalities originate. Here, I will summarize recent findings from the laboratory on DS and will discuss the possibility of early pharmacological intervention or *in utero* genetic manipulation in neuronal progenitors to recover brain development and cognitive deficits later in life in DS mice. In parallel, I will also discuss the possibility to develop safer (viral-free) technological approaches for genetic manipulations *in utero* to minimize technical issues in the view of potential translational applications in the far future.

The Brain-Boosting Power of Environmental Enrichment

Alessandro Sale¹

¹*CNR Neuroscience Institute, Pisa, Italy*

Brain plasticity refers to the remarkable property of cerebral neurons to modify their structure and function in response to experience. This fundamental theoretical theme has been inspiring a wealth of experimental work with a major focus on neural rehabilitation following brain disease. A very promising paradigm in this field is Environmental Enrichment (EE), consisting in an enhanced stimulation provided at multiple cognitive, sensory, and motor levels. Recent research performed at the Neuroscience Institute of CNR in Pisa has underscored a dramatic impact of EE on brain plasticity. Exposure to stimulating environments was shown to elicit, during development, a marked acceleration of brain maturation and, in the adult, resulted in the reopening of plasticity windows comparable to those characterizing juvenile sensitive periods. A common feature of this plethora of effects is the experience-dependent modulation of major regulators of neuronal plasticity, including the cerebral inhibition/excitation balance, the brain-derived neurotrophic factor (BDNF) and serotonin. The talk will present recent data concerning the influence of EE on brain plasticity, with major therapeutic potential for clinical application in neurological disorders characterized by compromised cerebral plasticity, such as amblyopia, Down syndrome and pathological brain aging.

Using time to generate a touch percept and touch to generate a time percept

Mathew E. Diamond & Tactile Perception and Learning Lab students, technicians

Cognitive Neuroscience, SISSA, Trieste IT

Research in the Tactile Perception and Learning lab at SISSA is based on three simultaneously acquired elements and the relation between them: 1. controlled, quantifiable sensory inputs, 2. measured behavioral output based on the subject's perception of the sensory input, 3. neuronal activity. The relation 1-2 is psychophysics; relation 1-3 is sensory coding; relation 2-3 is learning, memory, and decision making. Throughout the talk I will illustrate how the lab exploits this "golden triangle" in both humans and rats, focusing on the problem outlined below.

Sensory stimuli are frequently brief – just a fraction of a second – and can be composed of ambiguous streams of data. Experimental findings in primates, together with decision making models, have demonstrated that, in the face of such uncertainty, accumulation of information over time allows a more accurate estimate of the underlying external signal than does an instantaneous sampling of the input. Rodents, it was argued, use sensory "snapshots" rather than the more sophisticated mechanism of evidence integration.

We will show that, differently from earlier reports, rats can accumulate sensory information much like humans do. In the course of the study of temporal integration of touch signals, we made the surprising discovery that, in both humans and rats, the duration of a tactile stimulus affects its perceived magnitude: longer feels stronger. Neuronal recordings from behaving rats show that temporal integration, and the accompanying confusion between duration and intensity, occurs in the transformation from sensory cortex to frontal cortex.

If longer feels stronger, does stronger feel longer? We have developed duration estimation psychophysical tasks and found that, both in humans and rats, the intensity-duration perceptual confound is symmetric; yes – stronger feels longer. Future plans include further neurophysiology and optogenetics.

Human subjects who are healthy but at-risk for schizophrenia (assessed by familial occurrence) show an exaggerated intensity-duration perceptual confound, adding a new dimension to the well-known temporal dysperception in this syndrome.

All the experiments summarized above point to a major bug in sensory neuroscience. Neuroscientists always measure brain response by neuronal activity in units of time. Lab computers have a precise clock, but if the brain is using time to process its signals, what clock does it employ? Any timing mechanism in the brain could rely only on neuronal activity (that's all there is). And neuronal activity varies according to the ongoing stimulus. So the brain measures stimuli using time, which is modulated by the stimulus that evokes the activity. It's no wonder that humans and rats show a confound between stimulus percept and time.

ALTERED HEMISPHERIC ASYMMETRY OF LANGUAGE IN PSYCHOSIS: TESTING A THEORY WITH EVOLUTIONARY BASIS

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Timothy J. Crow first suggested that the genetic variance associated with the evolutionary development, in *Homo sapiens*, of language dominance in one hemisphere, is associated, in case of altered asymmetry, with the risk of failure manifest as the symptoms of schizophrenia (Crow, 1995). Therefore, only a small but relatively fixed fraction of individuals within a population will fail to develop the characteristic left hemisphere advantage for critical components of language as a consequence of an unfavorable genetic variation which leads to an increased risk to develop a psychotic disorder. The studies which will be presented started from the implementation of an EEG linguistic paradigm aimed at measuring language-related hemispheric lateralization and plasticity. Research carried out on hemispheric reorganization of language in aphasic patients and dyslexic children after linguistic training and recovery, allowed us to develop a connectivity model showing the linguistic hierarchical role of the left prefrontal region. The paradigm has been used as a tool to probe Crow's hypothesis and measure the loss of asymmetry between and within hemispheres in the schizophrenic brain. Recently, the paradigm has been used to further test Crow's hypothesis in other psychoses, by measuring language asymmetry in samples of patients affected by major depression and bipolar disorder.

THE YIN AND YANG OF MECHANOSENSING: A TALE OF TWO CHANNELS

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Biological processes as diverse as perception of sound and gravity, nociception, cell differentiation, and blood pressure regulation depend on the ability of our cells to detect mechanical cues from the environment and neighboring cells. Some of the molecular sensors for these cues are ion channels localized at the plasma membrane. Malfunction of mechanosensitive ion channels can lead to serious diseases, such as deafness, polycystic kidney disease, and xerocytosis. Novel examples of mechanosensitivity involving the Piezo1 and Hv1 channels in physiological and pathological conditions will be presented. Initial attempts to elucidate the molecular mechanisms underlying mechano-transduction mediated by these two channels will be discussed.

ADOLESCENCE VERSUS ADULTHOOD: DIFFERENCES IN BASAL DOPAMINE TRANSMISSION AND RESPONSE TO DRUGS OF ABUSE

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Adolescence is a crucial developmental period of important physiological, neurobiological and cognitive changes. This stage of life is particularly characterized by impulsivity and risk-taking and sensation-seeking behaviors, suggesting immaturity in decision-making processes. This developmental period corresponds also to a period of heightened vulnerability to drugs of abuse and development of addiction as well as to psychiatric disorders. Current theories attribute this behavioral pattern to differences in the maturation time course of cortical and sub-cortical areas and their coordination. Thus, there is evidence for a predominance of ventral striatum (approach system) relative to prefrontal cortex (regulatory system) that produce typical adolescent behaviors. Dopamine (DA) system is particularly involved in this stage of development since it is a key player in reward circuitries and incentive-motivated approach behavior, as well as in decision making processes. Although most of the studies suggest a delayed maturation of the prefrontal cortex (PFC), it is still debated if DA transmission in the nucleus accumbens (NAc) of adolescents is hyper- or hypo- reactive. Moreover, also the striatum appears to be involved in these typical adolescent behaviors.

In rodent models, in spite of overwhelming studies on reward function, tested through conditioned place preference or self-administration paradigms, direct evidence on adolescent DA transmission responsiveness to drugs of abuse is limited.

The aim of our study was to evaluate differences in mesolimbic and nigrostriatal DA transmission between adults and adolescents rats and its responsiveness to 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 or dorsolateral striatum (DLS) and challenged with nicotine, Δ^9 -tetrahydrocannabinol (THC), cocaine, or morphine and extracellular DA levels monitored simultaneously with behavior.

Although no significant difference was observed between adolescents and adults in basal DA levels, neither in the shell nor in the core of NAc, adolescents showed significant lower basal DA levels in DLS compared to adult rats. Different effects, depending on the drug and age of exposure, following drug administration were observed in adolescent and adult rats. While no difference was observed in DA transmission responsiveness, both in the shell and in the core of NAc, after cocaine administration, adolescent rats showed greater increase of extracellular DA in the NAc shell following nicotine, THC and morphine compared to adult rats. Moreover behavioral activation was significantly different in adolescent compared with adult rats.

While differences observed following THC and morphine might be explained by changes occurring in the endocannabinoid and opioid systems during development, differences following nicotine might be related to differential expression of nicotinic receptors at differential stages of development.

In conclusion these results while adding new insight in the development of the reward system during different stages of adolescence provide a likely explanation for the gateway effect of nicotine and THC toward abuse of other illicit substances.

ANOTHER CHAPTER OF THE “DRINKING IN THE DARK” SAGA: EXACERBATION OF ANXIETY UNDERLYING THE STRONGER URGE FOR ALCOHOL LATE AT NIGHT

Giancarlo Colombo, Carla Lobina, Carla Acciaro, Irene Lorrari, Paola Maccioni, Gian Luigi Gessa

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This lab has recently proposed a new animal model of binge-like drinking: selectively bred Sardinian alcohol-preferring (sP) rats consumed indeed up to intoxicating amounts of alcohol and displayed high sensitivity to time schedule when exposed to daily drinking sessions of one hour, occurring in the homecage with concurrent availability of three alcohol concentrations (0%, 10%, 20%, and 30%, v/v), and – most importantly – unpredictability of time of access to alcohol. Specifically, as the time of alcohol access moved over the dark phase of the 12:12 hour light/dark cycle, alcohol intake increased progressively; when the drinking session occurred at the end of the dark phase, alcohol intake averaged ≥ 2 g/kg, resulted in blood alcohol levels of ~ 100 mg% (meeting the criterion posed for binge drinking in humans), and produced severe motor-incoordination (*Alcohol* 48:301-11, 2014). Experiments of operant alcohol self-administration demonstrated that this sensitivity to time schedule extended also to the reinforcing and motivational properties of alcohol (*Psychopharmacology* 232:3585-95, 2015).

We hypothesized that the unpredictable schedule of alcohol access, together with the expectation of alcohol availability, may generate – as time elapses – a progressively increasing emotional “distress” in sP rats. The observed increase in alcohol intake might be interpreted as sP rats coping with this negative affective state by seeking the anxiolytic effect of alcohol. To verify this hypothesis, we designed two experiments aimed at evaluating whether (a) sP rats with an alcohol “history” comprising repeated, unpredictable exposures to multiple alcohol concentrations, in daily drinking sessions of one hour, displayed different levels of anxiety-related behaviors when exposed to the social interaction (SI) test at the first or last hour of the dark phase (Experiment 1) and (b) acute treatment with diazepam (a benzodiazepine with established anxiolytic profile) differentially affected alcohol drinking at the first and last hour of the dark phase in sP rats with an alcohol “history” comprising repeated, unpredictable exposures to multiple alcohol concentrations in daily drinking sessions of one hour (Experiment 2).

In Experiment 1, sP rats were exposed to the SI test the day after completion of a 12-day period of daily drinking sessions of one hour, during the dark phase of the light/dark cycle, with multiple alcohol concentrations, and unpredictable access to alcohol, during which alcohol intake positively correlated with the time of alcohol access [increasing progressively from 0.6 g/kg (first hour) to 2.1 g/kg (last hour)]. Data from the SI test indicated that time spent in SI activities (the shorter the time spent in SI activities, the higher the “anxiety” of the rats) was approx. 35% lower when the test occurred at the last rather than first hour of the dark phase.

In Experiment 2, sP rats were exposed to the pharmacological test with diazepam the day after completion of a 12-day period of daily drinking sessions of one hour, during the dark phase of the light/dark cycle, with multiple alcohol concentrations, and unpredictable access to alcohol, during which alcohol intake positively correlated with the time of alcohol access [increasing progressively from 0.7 g/kg (first hour) to 2.2 g/kg (last hour)]. Acute treatment with non-sedative doses of diazepam resulted in a dose-dependent decrease in alcohol intake in the rat group exposed to alcohol during the last hour of the dark phase; in comparison to vehicle treatment, alcohol intake was reduced by approx. 25%, 35%, and 50% in the rat groups treated with 1, 2, and 3 mg/kg diazepam. Conversely, diazepam treatment was completely ineffective in the rat group exposed to alcohol at the first hour of the dark phase.

Together, the results of Experiments 1 and 2 suggest the presence of higher anxiety-like states in sP rats at the last rather than first hour of the dark phase. These results may be interpreted as the “history” of unpredictable availability of alcohol producing an emotional “distress”, the intensity of which increased progressively over the dark phase, resulting – when alcohol was finally available – in proportionally larger intakes of alcohol. In other words, we hypothesize that sP rats may have coped with the negative affective state generated by the uncertainty of time of alcohol access with the observed increase in alcohol intake, seeking the anxiolytic effect of alcohol. This negative affective state, as well as the amount of alcohol consumed to cope with it, increased progressively over the dark phase together with alcohol expectation.

ELEVATION OF KYNURENIC ACID LEVELS SUPPRESSES DELTA⁹-TETRAHYDROCANNABINOL-INDUCED EXCITATION OF MESOLIMBIC DOPAMINE

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Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of *Cannabis* extracts, like most drugs of abuse, enhances dopamine (DA) transmission by increasing both DA neuron firing rate and DA release in the nucleus accumbens shell (shNAc). Moreover, THC enhances the firing rate of PFC pyramidal neurons projecting to the VTA. These effects, which are mediated by cannabinoid CB1 receptors, presumably underlie the rewarding and dependence-inducing effects of marijuana. Elevations of brain levels of kynurenic acid (KYNA), an L-tryptophan metabolite synthesized and released by astroglia in the brain, suppresses THC-induced behavioral and neurochemical effects, in rats and monkeys (Justinova *et al.* 2013).

On these bases, we carried out *in vivo* electrophysiological single cell recordings in anesthetized rats 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). According with previous studies, we confirmed that intravenously administered THC (0.3 – 2.4 mg/kg), increased firing activity of DA (137.1 ± 4.1 %, $n = 13$, $p < 0.05$) and mPFC (306.2 ± 75.6 %, $n = 6$, $p < 0.01$) cells projecting to the shNAc, as well as mPFC neurons projecting to the VTA (146.2 ± 26.7 %, $n = 7$, $p < 0.05$). 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. THC-induced increase in firing activity was completely abolished in DA (103.6 ± 3.3 %, $n = 7$) as well in mPFC cells projecting to the shNAc (119.4 ± 28.1 %, $n = 6$) and the VTA (72.6 ± 16.4 %, $n = 5$) recorded from rats pretreated with Ro ($p < 0.01$).

KYNA was suggested to act as a negative allosteric modulator of $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ -nAChRs), therefore, in the attempt to prevent Ro effects we administered positive allosteric modulators of $\alpha 7$ -nAChRs. Galantamine (3 mg/kg, *i.p.*), non-specific modulator of $\alpha 7$ -nAChRs, was unable to prevent the effects of Ro on VTA DA neurons, whereas PNU120596 (1 mg/kg, *i.p.* or *i.v.*), specific modulator of $\alpha 7$ -nAChRs, partially prevented the effects of Ro on mPFC pyramidal neurons, suggesting that the electrophysiological effects of KYNA might be dependent on $\alpha 7$ -nAChR. The involvement of $\alpha 7$ -nAChR was confirmed also with patch clamp experiments.

Patients seeking help for *Cannabis* dependence are increasing worldwide but specific pharmacological treatments are lacking and urgently needed, especially after the failure of CB1 antagonists due to psychiatric side effects. Our electrophysiological and neurochemical results support the hypothesis that specific modulation of KYNA levels might represent an innovative therapeutic approach to treat *Cannabis* dependence.

Reference: Justinova *et al.* 2013. *Nat Neurosci.* 16(11):1652-61.

ENDOCANNABINOID SIGNALING TO ASTROCYTES IN THE VENTRAL TEGMENTAL AREA AND MODULATION OF SYNAPTIC TRANSMISSION

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The mesolimbic dopamine system originating from the Ventral Tegmental Area (VTA) plays a prominent role in the cognitive processing of aversion, motivation, pleasure and reward. In different brain regions, including the basal ganglia, endogenous cannabinoids (eCBs) play significant roles in these processes by regulating excitatory and inhibitory transmission [1,2]. Given that the glial cell astrocytes have been reported to respond to eCBs with CB1 receptor-mediated Ca^{2+} elevations and, in turn, release gliotransmitters [1,3], we here advance the hypothesis of a direct contribution of astrocytes to the modulatory action of eCBs in VTA circuitry.

We combined Ca^{2+} imaging and patch-clamp recording techniques in VTA slices from young mice to address the following questions. Do VTA astrocytes respond with Ca^{2+} elevations to CBs? What types of gliotransmitters are eventually released by eCB-activated astrocytes? Do these gliotransmitters modulate VTA circuitry?

We observed that VTA astrocytes show somatic Ca^{2+} increases in response to WIN 55,212-2 mesylate (a CB1-CB2 receptor agonist) mediated by inositol-1,4,5-trisphosphate receptor type 2 (IP3R2) signaling pathway. We are currently performing electrophysiological experiments to study whether astrocytes activated by the release of eCBs affect excitatory transmission to VTA dopaminergic neurons. Initial results show that endogenous cannabinoids released by dopaminergic neurons upon depolarization - which act as a retrograde signal to inhibit homoneuronal synapses - potentiate excitatory transmission in adjacent heterosynapses. Interestingly, this potentiation is not observed in IP3R2 knockout mice, suggesting a prominent role of IP3R-mediated Ca^{2+} signals in this astrocytic action.

The modulation by astrocyte signaling of synaptic transmission in VTA circuitry opens a new perspective to the understanding of the complex cognitive processes in basal ganglia.

References:

- [1] Martin et al., Science 2015.
- [2] Alger, Progress in Neurobiology, 2002.
- [3] Navarrete and Araque. Neuron 2008.

KCC2 is a key target of oxytocin in postnatal events potentially involved in the pathogenesis of neurodevelopmental disorders

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Background Oxytocin (Oxt), a neurohormone known for its role in social behavior (Meyer-Lindenberg et al., 2011), has been implicated in the GABA switch in newborns (Tyzio et al., 2014). However, the molecular events involved in OXT regulation of the GABA switch were unknown.

Aims To elucidate how Oxt modulates the GABA switch and neuronal excitability in the first post-natal life, a critical period for neuronal maturation.

Methods We took advantage of oxytocin receptor null mice (*Oxtr*^{-/-}) that display an autistic-like phenotype which includes social and cognitive deficits and increased susceptibility to seizures, compatible with an altered E/I balance (Sala et al., 2011). The timing of the GABA was monitored in developing neuronal cultures by measuring GABA-induced Ca²⁺ responses, together with morphological, biochemical and electrophysiological analysis.

Results: Our data show that: i) *Oxtr* is necessary for the upregulation of the chloride cotransporter KCC2, a key player of the GABA switch, and for the correct timing of the GABA switch; ii) *Oxtr*, in a very early and narrow time window, directly modulates the functional activity of KCC2 by promoting its phosphorylation and insertion/stabilization at the neuronal surface; iii) in the absence of *Oxtr*, electrophysiological alterations are recorded in mature neurons

Conclusions: Our findings indicate that *Oxtr* is essential for the proper developmental increase of KCC2 and for the consequent switch in GABA activity. The identification of KCC2 as an Oxt target provides a better understanding of the role and therapeutic potential of Oxt for the treatment of neurodevelopmental disorders.

Leonzino M, Busnelli M, Antonucci F, Verderio C, Mazzanti M, Chini B. Oxytocin receptor regulates the timing of neuronal GABA switch via the Cl⁻ transporter KCC2, *Cell Reports*. 2016;15(1):96-103.

Sala M, Braida D, Lentini D, Busnelli M, Bulgheroni E, Capurro V, Finardi A, Donzelli A, Pattini L, Rubino T, Parolaro D, Nishimori K, Parenti M, Chini B. Pharmacological rescue of impaired cognitive flexibility, social deficits and increased aggression in the oxytocin receptor null mice, a neurobehavioral model of autism spectrum disorders. *Biol Psychiatry*. 2011 May 1;69(9):875-82.

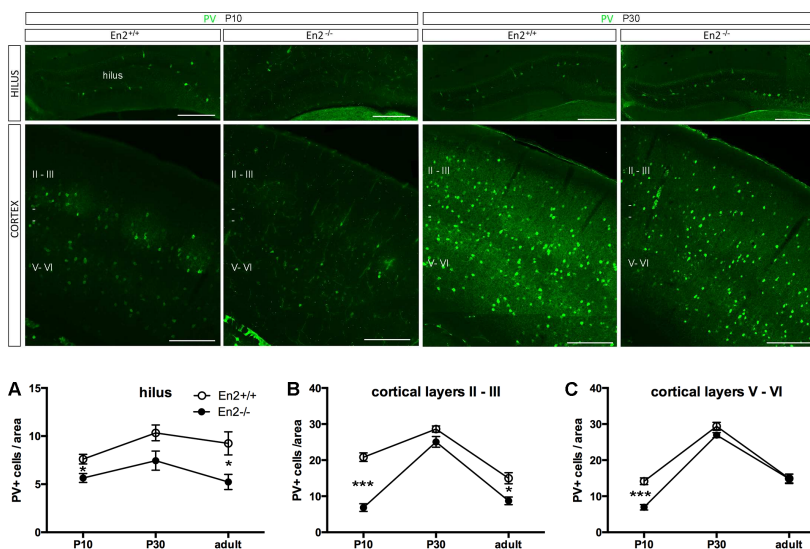
Tyzio R, Nardou R, Ferrari DC, Tsintsadze T, Shahrokhi A, et al. 2014. Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* 343:675-9

INTERNEURON DEFECTS DURING POSTNATAL DEVELOPMENT OF SOMATOSENSORY CORTEX OF ENGRAILED 2 KNOCKOUT MICE

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The homeobox-containing transcription factor Engrailed-2 (En2) is a candidate gene for autism spectrum disorder (ASD), and En2^{-/-} mice display anatomical and behavioural “autistic-like” features. Previous studies from our laboratory showed that adult En2^{-/-} mice have a reduced number of GABAergic interneurons in the hippocampus and sensory neocortical areas (somatosensory and visual). These defects are accompanied by increased seizure susceptibility, spatial learning deficits and a delayed maturation of visual function. Since reduced GABAergic inhibition has been proposed as a possible pathogenic mechanism of ASD, in this study we further investigated the postnatal maturation of inhibitory circuits in the En2^{-/-} somatosensory (S1) cortex. By using quantitative RT-PCR and immunohistochemistry, we showed that the expression of GABAergic interneuron markers parvalbumin (PV) and somatostatin (SST) is significantly reduced in the En2^{-/-} S1 cortex at postnatal day (P) 10, as compared to wild-type (WT) controls. This indicates that the interneuron defects observed in the adult En2^{-/-} neocortex have a developmental origin, and studies are ongoing to further characterize the anatomy of inhibitory circuits in the En2^{-/-} S1 cortex during postnatal development. Finally, to investigate the functional consequences of GABAergic interneuron defects in the En2^{-/-} S1 neocortex, we set up a battery of behavioural tests for the somatosensory function in adult mice. Our study will aim to establish a link between En2, anatomical deficits of GABAergic forebrain neurons and the pathogenesis of ASD.



PRENATAL EXPOSURE TO POLY I:C INCREASES SUSCEPTIBILITY TO EPILEPSY IN THE ADULT OFFSPRING

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It is increasingly appreciated that altered neuroimmune mechanisms might play a role in the development of neuropsychiatric disorders, including schizophrenia, autism spectrum disorder (ASD) and bipolar disorder. Within this neuroimmune framework of psychiatric diseases, a great deal of interest has been centered upon the possible contributions of infections in prenatal life. The prenatal period seems highly sensitive to the damaging effects induced by environmental insults such as infections. The accepted view is that prenatal infection, through a cytokine-dependent inflammatory status in the mother, can act as a "neurodevelopmental disease primer" responsible for a number of chronic mental illnesses.

In the last years, evidences have accumulated showing a direct connection between epilepsy and brain inflammation. Indeed, epilepsy is associated to enhanced inflammation, while activation of the immune response consequent to infections strongly increases the risk of seizures. Interestingly, recent evidence was provided demonstrating that prolonged inflammation during pregnancy is associated with increased hippocampal excitability in the offspring in an animal model.

By using the Poly I:C (polyinosinic-polycytidylic acid) mouse model of inflammation, we demonstrated that a single Poly I:C injection at gestation day 9 (GD9) is able to increase susceptibility to kainate-induced seizures in the offspring at postnatal day 90. As a control, mice injected with Poly I:C in the adult life show no increased susceptibility to kainate-induced seizures, thus providing the evidence that the higher susceptibility to seizures consequent to prenatal Poly I:C exposure is the consequence of a neurodevelopmental process.

We also evaluated the inflammatory profile of the Poly I:C-prenatally treated mice and we found that a single Poly I:C injection in early/mid gestation is able to induce an inflammatory response in the embryos 6 hours after the injection, as demonstrated by the significant increase of IL6 and IL1-beta mRNA levels. On the other hand, no significant signs of inflammation were found in adult mice, not even changes in synaptic protein levels and synaptic morphology indicating that these features, usually related to epilepsy, are not directly involved in the susceptibility to seizures in our model.

Although the mechanisms underlying this phenotype are still unknown, these results highlight the possibility that a single inflammatory event during pregnancy may alter neurodevelopment, leading to neurologic disorders in the adult offspring. Our results thus open to the possibility that inflammation during pregnancy may increase susceptibility to epilepsy by interfering with normal brain vascularization and BBB formation.

Combining Robotic Rehabilitation with silencing of the healthy hemisphere promotes True Motor Recovery in a Mouse model of Ischemic Injury

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Question: Stroke is one of the leading causes of chronic motor disabilities and re-acquisition of motor skills is crucial for stroke survivors. Animal models allow for a deeper understanding of spontaneous and induced post-stroke neuroplasticity and the testing of novel therapeutic strategies.

Methods: We used classical behavioral tests and an innovative kinematic analysis of the reaching movement (Lai et al., 2015) to investigate spontaneous evolution of motor deficits after photothrombotic stroke in the forelimb Primary Motor Cortex (Caudal Forelimb Area, CFA).

Moreover, post-stroke electrophysiological alterations of interhemispheric coupling in spared Premotor Cortex were studied by means of Field Potentials (FP) and Multi Unit Activity (MUA) recorded from the spared Rostral Forelimb Area (RFA) following optogenetic stimulation in the homotopic area of the healthy hemisphere in anesthetized transgenic mice.

We finally tested the efficacy of post-stroke rehabilitative strategies based on the application and the combination of robotic training (Spalletti et al., 2014) and transient inhibition of the healthy hemisphere with Botulinum Neurotoxin E (BoNT/E), intracortically injected in the homotopic contralesional CFA.

Results: We observed a robust functional impairment of the contralesional forelimb persisting at 30 days post-stroke and confirmed by altered kinematics of paw trajectories in the skilled reaching task.

In the RFA, we found a significant decrease of MUA, an increase of the hyperpolarizing component of the FP and of the Paired Pulse Inhibition after stimulation of the contralateral RFA. These alterations were specific for the ipsilesional hemisphere, indicating changes in interhemispheric functional connectivity after stroke and an increased inhibition exerted by the healthy hemisphere over the injured one. To probe the idea that altered transcallosal interactions hamper recovery from stroke, we reduced interhemispheric inhibition from the healthy to the injured side via delivery of the synaptic blocker BoNT/E. Remarkably, we found that coupling robotic rehabilitation with transient inhibition of the healthy hemisphere results in a functional improvement in general motor task and in kinematics of grasping, with re-establishment of pre-lesion movement patterns.

Conclusions: These data demonstrate the effectiveness of this experimental strategy in promoting true motor recovery. We are now studying the neural mechanisms at the basis of this recovery with electrophysiological, immunohistochemical and optogenetic tools.

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

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Multiple sclerosis is a complex neuroinflammatory disease whose pathogenic mechanisms involve an autoimmune activation against central nervous system (CNS) antigens. Peripheral activation is followed by immune cell infiltration in the CNS and biased interaction with resident CNS cells, including microglia and astrocytes. Multiple sclerosis develops mainly in a relapsing-remitting form, but most patients shift later to a (secondary) progressive form (while a minority show a (primary) progressive form from the beginning). The progressive form pathogenesis differ from the relapsing-remitting one: relapsing-remitting form drugs are unable to inhibit the evolution to the progressive one. This prompted to hypothesize that the pathogenic mechanisms triggering/sustaining the progressive forms are “brain-trapped” accompanied by a neurodegenerative process. Only exception is the very recent successful clinical trial with ocrelizumab (an anti-B lymphocyte monoclonal antibody) which is effective both in the relapsing-remitting (it reduces relapse rate) and primary progressive forms: however, the cognate drug rituximab (which also reduces relapse rate) was unable to inhibit the evolution from the relapsing-remitting to the secondary progressive form. These results suggest that the trigger (and perhaps the maintenance) of the progressive forms are partially dependent from “brain-resident” pathogenic mechanisms despite a common B lymphocyte-dependent mechanism. This hypothesis led us to look for molecules that, released inside CNS, would be able to act on both brain-resident and infiltrated immune cells and focused on calcitonin gene-related peptide (CGRP, a neuropeptide mainly synthesized by neuronal cells) that can be released both inside and outside the central nervous system to influence the activity of, for instance, microglia and astrocytes, but also immune cells.

To analyze the effect of CGRP in the development of experimental autoimmune encephalomyelitis (EAE), we released the peptide intrathecally by osmotic minipumps in a chronic fashion during the induction phase of EAE in mice: two days after EAE induction with MOG35-55 in C57BL/6 mice, we implanted a catheter (attached to an osmotic minipump) in a subdural location of the spinal canal (at the level of the VI vertebra). CGRP was delivered at 50 pmol/h and peptide administration lasted 15 days. Control mice received artificial CSF (aCSF). The animals were then perfused, spinal cord cut at a cryostat and sections processed for immunofluorescence: the results showed that the neuropeptide can dampen the disease course and change the shape of microglial cells (Sardi et al., 2014). We thus here analyzed the morphological and biochemical correlates of the CGRP action by using antibodies against microglia markers (Iba1 or CD11b), astrocyte markers (GFAP), iNOS (M1-type macrophage/microglia activation marker), Ym1 (M2-type macrophage/microglia activation marker), CD206, CD68, phosphorylated ERK1/2, phosphorylated p38. In brief, the analysis of the 3D structure of the ramifications of microglia processes was performed along the whole length of the spinal cord, but restricting the analysis to its ventral and dorsal median aspects (the areas which are more consistently infiltrated). By expanding the findings of the previous 2D analysis, a 3D analysis showed that the microglia ramifications also were changed by CGRP treatment. Moreover, the analysis of meningeal (pia mater) infiltration in the same areas showed a significant reduction in the number of infiltrated cells. In addition, a reduction in the percentage of Ym1-positive cells among infiltrated cells was observed. Finally, since CGRP was shown to mediate tolerance to morphine-induced analgesia by activation of ERK (in astrocytes) and p38 (in microglia) in the spinal cord, we analyzed the same intracellular cascade: during EAE development the protective effect of CGRP was associated with ERK activation in astrocytes, but not p38 activation in microglia.

The present results show that CGRP protective effect during EAE induction is associated to reduced infiltration, changes in microglial cells ramifications and ERK activation in astrocytes, but not p38 activation in microglia. This shows that the peptide acts in a context-dependent, cell-specific manner. Moreover, CGRP protective effect is not mediated by the so-called “beneficial” activation of microglia (exemplified by M2-activation), but rather by a homeostatic action that dampens any activation, possibly driving toward a “more resting” state.

Targeting retinal inflammation to delay photoreceptor degeneration in Retinitis Pigmentosa

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In Retinitis Pigmentosa (RP), a genetic defect causes the primary degeneration of retinal rods and night blindness. Due to non-cell autonomous, bystander effects, cones also die secondarily and blindness occurs. Genetic variability is a hallmark of RP, with hundreds known mutations in over 60 different genes identified so far. Searching for biological features common to different forms of the disease is crucial for developing effective therapeutic strategies.

Previous data from our group show that Environmental Enrichment (EE) from birth is effective in slowing down photoreceptor degeneration in a mouse model of RP, considerably expanding the time windows of effective vision. Searching for the molecular effectors of the protective retinal response triggered by EE exposure, we used Next Generation Sequencing followed by qRT-PCR analysis to compare gene expression in degenerating retinas exposed to EE and retinas of standard raised mice. We found that retinal degeneration results in a major inflammatory and immune response at retinal level, albeit a general assumption exists that RP is not an inflammatory disease. To test the hypothesis that EE rescue effect could be mimicked by an anti-inflammatory treatment, we implemented a protocol of chronic dexamethasone (DEXA) administration on standardly raised, rd10 mutant mice, a well-established model of human RP.

Groups of rd10 mice received daily Dexamethasone (Soldesam forte 4mg/ml), administered with a gavage needle, in a dosage progressively decreasing between 3-0,75 mg/Kg. rd10 control mice were given water; each group was treated from P23 to P45. For histological studies, retinal whole mounts were fixed in 4% PFA and stained with cell-specific antibodies for cone photoreceptors (cone opsins) and microglia (Iba1), then imaged by fluorescence and confocal microscopy for cell population counts.

We found that DEXA treated groups showed an increased number of surviving cones compared to matched controls. The anti-inflammatory effect of the drug was confirmed by a decreased number of retinal microglial cells displaying an amoeboid, activated, state. This effect had been previously found in retinas of EE rd10 mice. Molecular studies are ongoing to confirm a reduced global pattern of retinal inflammation in DEXA treated retinas.

These data open the perspective of slowing down retinal decay in human RP subjects by using dexamethasone or related drugs. Indeed, a sustained release device based on this molecule is already in use for macular edema and its repurposing for RP (an otherwise orphan disease) should be easily achievable.

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REGENERATION OF THE PARALYZED NEUROMUSCULAR JUNCTION

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The NeuroMuscular Junction (NMJ) is the anatomical structure where motor neurons axon terminals (MAT) deliver its stimulus to the interfacing muscle fibers (MF). A multicellular layer of perisynaptic Schwann cells (PSC) covers the MAT to seal the synapse and preserve its integrity. At variance from central synapses, NMJs are not protected by anatomical barriers and are exposed to many types of damage, including mechanical traumas and the attack of many pathogens. Accordingly, vertebrates have evolved the capacity to restore NMJ integrity and functionality following different kinds of injury, including the ability to regenerate and remodel the synapse. PSC seem to play a major role as they physically drive MAT regrowth and guide synaptic remodelling. MF also contributes with some nuclei strategically positioned underneath the NMJ and which are fully dedicated to the synapse. A precise orchestration of intracellular and intercellular signals is therefore expected to occur among the injured MAT, PSC and MF during NMJ regeneration and remodelling, but little is known about the underlying cellular and molecular events. Here we use two well-characterized NMJ pathogens, i.e. Botulinum neurotoxins (BoNTs) and α -Latrotoxin (α -LTX) to trigger and study the molecular crosstalk underpinning two paradigmatic responses of the NMJ: the extensive synaptic remodelling induced by the prolonged functional impairment caused by BoNTs, and the process of degeneration/regeneration induced by α -LTX. By analysing the time resolved transcriptomes of intoxicated NMJs, we found that the chemokine CXCL12 is produced by PSC following the acute degeneration of the MAT and its signalling through the CXCR4 receptor is fundamental for NMJ regeneration.

iPSC-based in vitro model of Parkinson's disease caused by OPA1 mutations

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New emerging evidences link neurodegenerative disorders, such as Parkinson Disease (PD) and Parkinsonism, with mitochondrial dysfunction, focused on quality control and mitochondrial dynamics (e.g. fission and fusion). The OPA1 gene encodes for a dynamin-related protein of the inner mitochondrial membrane that regulates mitochondrial fusion and cristae remodelling. OPA1 mutations are associated with dominant optic atrophy. However, recently, new OPA1 missense mutations were reported in patients with age-related PD and cognitive impairment. These mutations affects highly conserved amino acids (p.G488R, p.A495V) in the GTPase domain of the protein. We reprogrammed skin fibroblasts into transgene-free iPSCs from three independent patients and unrelated healthy donors. hiPSCs were then differentiated into stable neural progenitor cell (NPC) lines and dopaminergic neurons. Interestingly, OPA1 mutant NPCs showed in basal conditions a general reduced content of mitochondria with a more fragmented morphology compared to control cells. Moreover, OPA1 mutant NPCs exhibited a net decrease in cellular respiration, heightened oxidative stress and overall reduction in mitochondrial DNA content. Finally, in glucose-deprived medium, OPA1 mutant, but not control, NPCs had reduced proliferation and vitality. Importantly, reintroduction of the OPA1 functional gene was sufficient to rescue mitochondria dysfunctions in patients' cells. NPCs have been differentiated into dopaminergic neurons and tested for mitochondrial homeostasis and function as well as for vitality in basal and stress conditions. These experiments will unveil the OPA1-dependent molecular mechanisms of neurodegeneration and possibly the bases for the accentuated vulnerability of dopaminergic neurons. This study establishes a new human *in vitro* model for neurodegenerative disorders ideal for revealing the relation between mitochondrial dysfunctions and neuronal death as well as particularly attractive for developing new approaches of neuroprotection and translational therapies.

TSPAN5 IS A KEY PLAYER IN DENDRITIC SPINE FORMATION AND AMPA RECEPTOR RECYCLING

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TSPAN5 is a brain-enriched protein member of the tetraspanin superfamily. These proteins have the peculiar ability to form specialized membrane region called Tetraspanin-Enriched Microdomains (TEMs) where they accumulate other proteins.

We found that TSPAN5 is present at the cell membrane in developing hippocampal neurons while it is concentrated in intracellular post-synaptic compartment in mature neurons. We hypothesized that these age-dependent localisations could reflect different functions.

To address the first putative function in younger neurons we knocked down TSPAN5 and found a dramatic reduction in dendritic spines number. We observed, in developing hippocampal neurons, that the synaptic adhesion-molecule Neuroligin-1 and the AMPAR subunit GluA2, fundamental for dendritic spines formation and stabilization respectively, were associated with TSPAN5-dependent TEMs. Through a novel developed high-resolution single-molecule tracking technique called uPaint, we found that knockdown of TSPAN5 led to increased mobility of Neuroligin-1 and GluA2 suggesting a loss of the clustering typical of dendritic spines genesis. We hypothesized that TSPAN5, through the organization of TEMs, could be responsible for dendritic spines formation by trapping these proteins in specific membrane locations.

To understand the second intracellularly exerted function of TSPAN5 we used a yeast two-hybrid screening on the C-terminal tail of the protein and identified AP-4 complex as a new interactor of TSPAN5. This complex is known to regulate AMPARs trafficking by binding the auxiliary subunit Stargazin.

We observed that the knockdown of TSPAN5, in mature neurons, caused a strong decrease in surface GluA2 levels, due to increased lysosomal degradation. Other evidences suggested the presence of TSPAN5 in recycling endosomes in mature neurons.

We demonstrated that TSPAN5 is necessary for the correct recycling of GluA2-containing AMPA receptor in basal condition.

These results highlight multiple roles of TSPAN5 in regulation of synapse formation and synaptic functioning through two distinct mechanisms of action.

AUTOPHAGY INDUCTION BY THE NATURAL COMPOUND PTEROSTILBENE: MECHANISMS AND THERAPEUTIC POTENTIAL

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Pterostilbene (Pt) is a natural dietary phenol found in berries that exhibits, at least *in vitro*, a variety of bioactivities of potential relevance. Several studies suggest that Pt may have protective effect vs. neurodegeneration and that it can improve cognitive performance deteriorated by age. Most importantly, this molecule shows an high bioavailability. Indeed, μM -range concentrations of Pt were detected in the organs of rats after a single intragastric administration (88 $\mu\text{moles/Kg}$) (Azzolini et al., 2014). However, although the beneficial effects of this molecule are widely recognized, its molecular mechanisms of action are still undefined. Up-regulation of autophagy may be one of these mechanisms.

Autophagy is a cellular catabolic process activated for protection/regeneration by cells in response to some stressing conditions and it is mainly regulated by Transcription Factor EB (TFEB). Its translocation to the nucleus and its activity depend on phosphorylation/dephosphorylation events mediated by mTORC1 kinase and the Ca^{2+} -dependent phosphatase Calcineurin.

A pro-autophagic role has been already ascribed to stilbenoids. Therefore, we wanted: 1) To verify whether Pt could induce TFEB migration; 2) In case of a positive answer to the 1st point, to investigate the molecular mechanism behind this phenomenon.

HeLa cells overexpressing TFEB-GFP were treated with physiologically meaningful concentrations of Pt or of two of its major metabolites and monitored by confocal microscopy for up to three hours. As hypothesized, Pt induced TFEB migration to the nucleus in our system but to a lesser extent than nutrients deprivation. Accordingly, Pt decreased the phosphorylation levels of S6, a mTORC1 target. Moreover, while the sulfated form, a phase II metabolite, was almost ineffective, DiHydroPt produced by gut microbiota showed an activity similar to that of the parent compound, but required higher concentrations. Finally, autophagy onset was confirmed through RT-qPCR showing an up-regulation of some selected TFEB target genes as well as by Western Blotting analysis reporting an increase in the lipidation of LC3B.

The implication of cAMP/AMPK was evaluated by using a FRET sensor measuring cAMP and treating HeLa cells overexpressing TFEB-GFP either with A769662 (a known AMPK activator) or IBMX (a pan-inhibitor of phosphodiesterases). Pt provoked a modest but significant increase in cAMP concentration in HeLa cells. This may be ascribed to partial inhibition of phosphodiesterases, which was indeed observed in ad hoc assays. Interestingly, treatment with A769662 or IBMX induced a less pronounced TFEB translocation into the nucleus than the one induced Pt. These observations lead us to speculate that our compound might induce TFEB nuclear translocation also by modulating other signaling pathways.

In light of our *in vitro* data, we tested Pt as a potential therapeutic treatment for Collagen VI (ColVI) muscular dystrophies characterized by a defective autophagic flux and by the accumulation of damaged organelles. To this purpose, we took advantage of a zebrafish model of Collagen VI related myopathies obtained by injecting an antisense morpholino specifically directed against ColVI exon 9 splicing region. Preliminary results indicate that, while Pt treatment induced a recovery of muscular structure by more than 30%, it had only mild effects on the recovery of motor activity of the morphants characterized by a strong impairment of the organization of the myofibers.

Azzolini M, La Spina M, Mattarei A, Paradisi C, Zoratti M, Biasutto L.
Pharmacokinetics and tissue distribution of pterostilbene in the rat.
Mol Nutr Food Res. 2014; 58:2122-32.

The cell surface trafficking of the $\alpha 3\beta 4$ neuronal nicotinic acetylcholine receptor is regulated by its accessory subunit

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Neuronal nicotinic acetylcholine receptors are a large family of cationic channels consisting of nine α ($\alpha 2$ - $\alpha 10$) and three β subunits ($\beta 2$ - $\beta 4$) which assemble in pentamers with different subunit composition. The $\alpha 3\beta 4$ receptors may be present in two alternative stoichiometries: $2\alpha/3\beta$ and $3\alpha/2\beta$ that have similar agonist sensitivity but different antagonist sensitivity, and markedly different single-channel conductance. Recently, it has been shown that, in heterologous systems, nicotine, the prototypical agonist of these receptors, upregulates the intracellular and plasma membrane $\alpha 3\beta 4$ receptors. Nicotine privileges the assembly of $(\alpha 3)_2(\beta 4)_3$ pentamers which are more efficiently delivered to the cell surface because of the presence in the β subunit of an endoplasmic reticulum export motif (LXM). In order to dissect the mechanism by which the receptor reaches to plasma membrane, we generated a dimeric construct ($\beta 4$ - $\alpha 3$) that, when co-transfected with a monomer ($\alpha 3$ or $\beta 4$), allows to study a specific population of receptors with fixed stoichiometry. By means of morphological, biochemical and functional assays, we found that after 24 hour transfection only the receptors with three $\beta 4$ subunits are recruited to plasma membrane and, very importantly, that the type of accessory subunit determines the plasma membrane trafficking. Indeed, even if $\beta 4$ in the dimer is mutated in the LXM motif, the presence of the wild-type $\beta 4$ in the fifth position allows a regular trafficking of the receptor to the cell surface. This study demonstrates a novel function of the accessory subunit in the $\alpha 3\beta 4$ receptor that may be relevant also for other pentameric receptors.

TYPE I DIABETES MICROVASCULAR COMPLICATIONS AND INTERACTION WITH HLA: A LINKAGE ANALYSIS

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Detecting gene-gene interaction in complex diseases has become an important priority for common disease genetics, but most current approaches to detect interaction start with disease-marker associations. We developed, tested and successfully applied a powerful linkage-analysis-based gene-gene interaction method. Our detection strategy is based on conditioning the family data on a known disease-causing allele or disease-associated marker allele (as would have been determined from a previous analysis). We computer-generated multipoint linkage data for a disease caused by two epistatically interacting loci (A and B) and examined several two-locus epistatic inheritance models: dominant-dominant, dominant-recessive, recessive-dominant, recessive-recessive. The family data were pruned based on the presence of the known disease-related allele (A), and the family members who did not carry this allele were removed from the analysis (we referred at this analysis as stratified). This elimination step has the effect of raising the “penetrance” and detectability at the second locus (B). We used the lod scores for the pre- and post-stratification data sets to calculate a statistic that either indicated the presence of interaction or indicated that no interaction was detectable. Moreover, we examined if the presence of interaction with locus B could be detected based on a disease-marker association at locus A and if the presence of genetic heterogeneity would generate false positive evidence of interaction.

We applied this method to test the association of HLA alleles and type 1 diabetes (T1D) microvascular complications, given that HLA alleles are associated with T1D, but the association with its microvascular complications is still controversial.

The HLA association with retinopathy and nephropathy was tested in 415 multiplex T1D families from the Human Biological Data Interchange (HBDI) collection by Single Nucleotide Polymorphisms (SNP) markers spanning on chromosome 6. In a previous work, we performed logistic regression models (adjusting for sex, age of T1D diagnosis and duration of T1D) among T1D probands, defining ‘cases’ patients with complications and ‘controls’ complications-free patients. From these analyses a strong association was found between T1D complications and both DRB1*03:01 and DRB1*04:01 alleles.

To test possible alleles’ interaction, our novel methodology was applied and the stratification of the subjects was based on these DRB1*03:01 and DRB1*04:01 T1D associated alleles. When analysing the DRB1*03:01-positive retinopathy families, in addition to the novel telomeric locus from our earlier work, one centromeric to HLA was identified at the same location as the nephropathy peak. When we stratified on DRB1*04:01-positive families, the HLA telomeric peak strengthened but the centromeric peak disappeared.

Our results show that in T1D patients, specific HLA alleles may be involved in susceptibility to, or protection from, microvascular complications, suggesting an interaction between specific HLA alleles and other loci that influence complications’ expression.

MONOAMINE OXIDASES CAUSE MITOCHONDRIAL AND ENDOPLASMIC RETICULUM STRESS LEADING TO CARDIOMYOPATHY IN TYPE 1 DIABETES

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Monoamine oxidases (MAOs) play a major role in the oxidative stress and cardiovascular damage. Reactive oxygen species (ROS) and inflammation are major contributors to the development of diabetic cardiomyopathy (DCM), but so far the involvement of MAO in these processes has been overlooked. Here we investigated whether MAOs contribute to oxidative stress, mitochondrial dysfunction and endoplasmic reticulum (ER) stress in diabetes-induced cardiac dysfunction and remodeling. Neonatal rat ventricular myocytes displayed a significant increase in mitochondrial ROS formation and loss of mitochondrial membrane potential when exposed to high glucose (HG, ≥ 1.3 fold, $p < 0.05$) or a combination of HG and interleukin- 1β (IL 1β , ≥ 2.7 fold, $p < 0.05$), a pro-inflammatory cytokine found to be elevated in diabetes. MAO inhibitor pargyline reduced ROS formation in both conditions, suggesting that HG and IL 1β induce oxidative stress in a MAO-dependent manner. Both HG and IL 1β triggered ER stress, as evidenced by an increase in phospho-IRE1 α levels and protein expression of ER stress markers ATF4, GADD34, CHOP and GRP78. Pargyline treatment prevented this, suggesting that MAO activation and ROS formation are upstream of ER stress, at least in these conditions. In the *in vivo* model of type 1 diabetes (T1D), pressure volume loops analysis showed that diastolic stiffness, an index of diastolic dysfunction, was increased ~ 4.6 fold in T1D mice. Pargyline administration to T1D mice reduced diastolic stiffness by 50% thus preventing diastolic dysfunction. Moreover, oxidative stress, ER stress and fibrosis were increased in T1D hearts. Pargyline prevented these events, indicating that MAO contributes to cardiac damage and dysfunction in diabetes. The present study suggests that, in addition to mitochondria, MAO-generated ROS are also able to target neighboring organelles, such as ER. MAO inhibition prevents structural and functional changes triggered by T1D in the heart, thus representing an attractive therapeutic strategy for the treatment of DCM.

The unique histidine of F-ATP synthase subunit OSCP mediates regulation of the permeability transition by matrix pH

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The mitochondrial permeability transition pore (PTP) is a Ca²⁺- and oxidant-dependent, large conductance channel of the inner membrane, whose prolonged opening leads to cell death. Its molecular nature has been long debated and numerous protein candidates have been proposed that did not stand the test of genetics. The recent discovery that F₀F₁ ATP synthase can form the PTP under conditions of high Ca²⁺ and oxidative stress poses the challenging opportunity to define the properties of the PTP based on the structural properties of ATP synthase [1].

Pioneering studies established that in rat and mouse liver mitochondria the probability of PTP opening has an optimum at neutral matrix pH, while the pore is blocked at acidic pH due to reversible protonation of PTP histidyl residue(s). With the goal to identify the ATP synthase histidyl residues involved in the pH modulation of channel formation, we focused our attention to the OSCP subunit, because it strongly influences the threshold Ca²⁺ required for PTP opening. Replacement of the unique conserved histidine of OSCP (His112 -bovine numbering) with a Gln (H>Q) in HEK293T cells provided evidence that His112 is responsible for the PTP block at acidic pH. Indeed, in wild type cells PTP opened at pH 7.4, while it was strongly inhibited at pH 6.5. Conversely, in H>Q cells Ca²⁺ addition induced channel formation at both neutral and acidic pH. Moreover, the histidine-modifying DPC was effective in allowing PTP opening at acidic pH in wild type cells, while in H>Q cells DPC had no effect both at neutral and acidic pH. These data provide compelling evidence that conformational changes of OSCP located at the F₁ top can affect the propensity of channel formation in the more 100 Å distant inner membrane.

1. P. Bernardi, A. Rasola, M. Forte, G. Lippe, The Mitochondrial permeability transition pore: channel formation by F-ATP Synthase, integration in signal transduction, and role in pathophysiology, *Physiol Rev* 95 (2015) 1111-55.