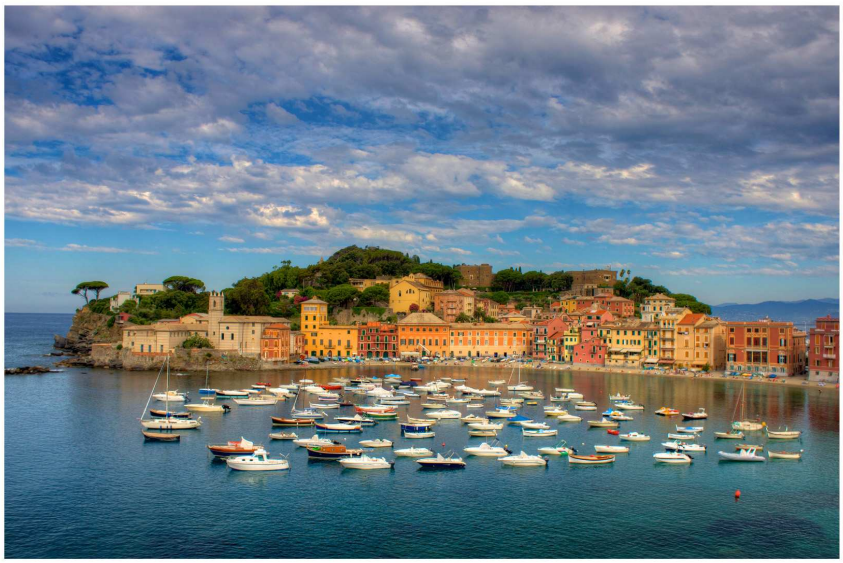
6th Meeting
Young Researchers in Physiology

Fondazione MediaTerraneo
Sestri Levante (GE) – May 30-June 1, 2012



6th Annual Meeting of Young Researchers in Physiology
Sestri Levante May 30 – June 1, 2012

SHORT PROGRAMME

Wednesday May 30

11.00-13.00 Registration of the participants and poster installation
13.00-14.00 Lunch
14.30-15.00 Opening ceremony
15.00-16.00 **Keynote Lecture I: "Aquaporins in physiology and disease" (Maria Svelto)**
16.00-16.30 Coffee break
16.30-19.00 **Oral communications:** ***Neurophysiology I***
20.00 Free dinner (ticket)

Thursday May 31

09.00-11.00 **Oral communications:** ***Cell Physiology I***
11.00-11.30 Coffee break
11.30-13.00 **Guided Poster Visits**
13.00-14.00 Lunch
14.00-15.30 **Round Table: "ANVUR: recruitment and evaluation of scientific productivity in Italy"**
15.30-17.00 **Poster discussion I:** ***Neurophysiology***
17.00-17.30 Coffee break
17.30-18.30 **Keynote Lecture II: "Three-dimensional super resolution microscopy with focused light" (Alberto Diaspro)**
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Friday June 1

09.00-10.00 **Poster discussion II:** ***Cell physiology – Muscle physiology***
10.00-10.30 Coffee break
10.30-13.00 **Oral communications:** ***Neurophysiology II***
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14.00-15.00 **Round Table: "The future in research: grant opportunities for young investigators"**
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17.30-18.00 Coffee break
18.00-19.30 **Keynote Lecture III: "Brain plasticity and the influence of the environment" (Lamberto Maffei)**
20.00 **Social Dinner & Award Ceremony**
22.00 **Concert: "Something Gnu"**
The GnuQuartet: Francesca Rapetti (flute), Roberto Izzo (violin), Raffaele Rebaudengo (viola), Stefano Cabrera (cello)

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Maria Svelto, Department of Biosciences, Biotechnologies and Pharmaceutical Sciences, University of Bari

16.00-16.30 Coffee break

16.30-19.00 **Oral communications:**

Neurophysiology I

Federica Bosco (University of Genova): The tyrosine phosphatase STEP modulates synaptic function at the presynapse.

Manuela Fadda (The Italian Institute of Technology, Genova): Synapsin II mutations associated with autism or epilepsy: effects on neuronal development and synaptic vesicle cycling.

Antonella Marte (University of Genova): LRRK2 interacts with synaptic vesicles at the presynaptic level.

Laura Perlini (The Italian Institute of Technology, Genova): Synapsin III regulates radial migration and morphological maturation of pyramidal neurons *in vivo*

Chiara Criscuolo (University of L'Aquila): Brain-derived neurotrophic factor (BDNF) synaptic function and dysfunction: focus on beta amyloid toxicity.

Michele Bertacchi (Scuola Normale Superiore, Pisa): The positional identity of mouse embryonic stem cells-generated neurons is affected by BMP signaling.

Eva Terzibasi Tozzini (Scuola Normale Superiore, Pisa): Adult neurogenesis and age-modulated microRNA expression in the brain of the short-lived teleost *Nothobranchius furzeri*, an emerging model for ageing studies.

Antonio Falace (University of Genova): TBC1D24: a novel epilepsy gene involved in cortical network development.

Carlo Natale Giuseppe Giachello (University of Torino): Pentylenetetrazole-induced epileptiform activity impairs basal synaptic transmission and short-term plasticity in monosynaptic connections.

Lucia A. Ruocco (Second University of Napoli): Higher density of NMDAR1 subunit in prefrontal cortex of an animal model of hyperactivity and attention deficit: expression, functional relevance and plasticity.

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Cell Physiology I

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Debora Landi (University of Pisa): miR-361 cooperates with SRIF to prevent hypoxia-induced accumulation of VEGF in HUVEC.

Maria Cristina Mantovani (University of Milano): Schwann cells and mesenchymal stem cells as functional tool promoting peripheral nerve regeneration.
Sara Maria Fossati (The Italian Institute of Technology, Genova): Characterization of the Octopus arm morphology and its regenerative capability: role of Acetylcholinesterase during morphological modification.
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Piergiorgio La Rosa (University of Roma Tre): 17 β-Estradiol modulation of cell proliferation requires diverse post-translational modification of estrogen receptor α.
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14.00-15.30 **Round Table: "ANVUR: recruitment and evaluation of scientific productivity in Italy"**
Giuseppe Novelli, ANVUR, Member of the Board of Directors; Director of U.O.C. Medical Genetics Laboratory, Tor Vergata University, Rome and Adjunct Professor, University of Arkansas for Medical Sciences, USA; **Mauro Degli Esposti**, Lecturer, University of Manchester, UK; ANVUR, Member of the GEV Biological Sciences; Founder of the Virtual Italian Academy (VIA-Academy).

15.30-17.00 **Poster discussion I:**
Neurophysiology
Marta Orlando and Erica Tagliatti (The Italian Institute of Technology, Genova): Synapsin function in the regulation of the synaptic vesicle cycle in presynaptic terminals of hippocampal neurons.
Shahrzad Latifi (The Italian Institute of Technology, Genova): Presynaptic targeting of optogenetic probes.
Oscar Gerardo Brenes Garcia (University of Torino): *In vitro* monosynaptic circuits of *Helix* neurons as an experimental model to study synapsin knock-down.
Placido Illiano (The Italian Institute of Technology, Genova): Generation of humanized Dopamine Transporter Knock-in mouse strains carrying loss-of-function mutation as a model of human Dopamine Transporter Deficiency Sindrome.
Luisa Speranza (University of Napoli Federico II): Serotonin receptor 7 (5-Htr7): a key-regulator of neurite outgrowth in telencephalic neurons.
Roberto Ripa (Scuola Normale Superiore, Pisa): Genetic methods to manipulate brain aging in the short-lived fish Nothobranchius furzeri: an novel model species for aging studies.
Nicola Maria Carucci (Scuola Normale Superiore, Pisa): The contribution of early inflammation to neurodegeneration in anti-nerve growth factor mice.
Marco Barbariga (University of Milano): Cerebrospinal fluid ceruloplasmin oxidation induces NGR motifs deamidation with gain of pro-adhesive function.
Gabriele Deidda (The Italian Institute of Technology, Genova): Early-depolarizing GABA controls critical period plasticity in the rat visual cortex.

Alessandro Arena (Vita-Salute San Raffaele University, Milano): Analysis of the effects of anesthetics on the activity of rat visual cortex.
Elisa Castaldi (University of Firenze): Selectivity to spatial phase of chromatic cortical mechanisms: an fMRI study.
Giulia D'Urso (The Italian Institute of Technology, Genova): Role of layer V principal neurons in the regulation of the integration property of cortical columns in the mouse somatosensory cortex.
Claudia Fuchs (University of Bologna): APP-dependent neurogenesis impairment of neural precursors from the Ts65Dn mouse, a model for Down sindrome.
Fiorenza Stagni (University of Bologna): Dendritic pathology and connectivity can be rescued by pharmacotherapy with fluoxetine in the Ts65Dn mouse model of Down sindrome.
Thorsten Becker (University of Verona): Endocannabinoids control the GABA-ergic neurotransmission on orexinergic neurons in the lateral hypothalamic area in obese mice.
Elisa Tavazzani (University of Pavia): Electrophysiological evidence for potassium accumulation between type I hair cells and calyx terminals in mammalian crista ampullaris.
Concetta Treno (Second University of Napoli): A neurogenetic approach to the study of non spatial attention in mice and rats.
Federico Del Gallo (University of Milano): A mouse model of fatal familial insomnia (FFI): REM sleep reduction in transgenic mice.
Marco Segatto (University of Roma Tre): The regulation of emotional aspects of behaviour: a new role for cholesterol biosynthetic pathway in the central nervous system.
Matteo Fecchio (University of Milano): Slow waves evoked by transcranial magnetic stimulation reflect a cortical downstate.
Andrea Pigorini (University of Milano): Neuronal downstates and cortical breakdown of causality during NREM sleep: an intracerebral study in humans.

17.00-17.30 Coffee break

17.30-18.30 **Keynote Lecture II: "Three-dimensional super resolution microscopy with focused light"**
Alberto Diaspro, Nanophysics Department, The Italian Institute of Technology, Genova

20.00 Free dinner (ticket)

Friday June 1

09.00-10.00 **Poster discussion II:**

Cell physiology – Muscle physiology

Serena Milano (University of Bari): Statins treatment increases AQP2 plasma membrane expression in vitro and in vivo: potential usefulness in the treatment of nephrogenic diabetes insipidus (NDI).
Silvia Torretta (University of Bari): NKCC2 trafficking and activity: the role of interacting proteins.
Melania Melis (University of Cagliari): Do salivary proteins influence bitter taste to 6-n-propylthiouracil (PROP) in humans?
Giovanna Trinchese (University of Napoli Federico II): Diet supplementation with donkey milk up-regulates liver mitochondrial uncoupling, reduces energy efficiency and improves anti-oxidant and anti-inflammatory defences in rats.
Sheila Maria Alvarez Fernandez (University of Milano): Surface ADAM10 protein elicits a serological immune response in colorectal cancer patients.

Eleonora Margheritis (University of Insubria): Characterization of lysine - containing dipeptides transport by different PepT1 isoforms expressed in *Xenopus laevis* oocytes.
Alessandra Vollero (University of Insubria): Relationship between temperature and kinetic properties in rabbit intestinal oligopeptide cotransporter PepT1.
Emanuele Murana (University of Roma La Sapienza): Transient increase in neuronal chloride concentration by soluble factors released from glioma cells.
Marina Angelini (University of Milano): Positive correlation between CLIC1 expression in the plasma membrane and human glioma aggressiveness.
Rosaliana Libro (University of Pisa): Role of hypoxia in the regulation of microvesicles-derived microRNAs in colorectal cancer cells.
Annarita Di Mise (University of Bari): Constitutively active variants of the calcium-sensing receptor: parallel adaptive feedback to explain the molecular basis of the gain of function.
Valeria Rossetti (University of Milano): Oxidative stress of respiratory cells induced by H2O2 or cigarette smoke extract is reduced by the subministration of S-CMC-Lys.
Adriana Carol Eleonora Graziano (University of Catania): Molecular mechanisms involved in psychosine-induced apoptosis.
Francesca Tullio (University of Torino): Cardioprotection by postconditioning in experimental models of cardiac hypertrophy: spontaneously hypertensive and nandrolone-abuse rats.
Marco Pellegrini (University of Roma Tre): Does a different susceptibility to endocrine disruptors between sexes exist? The example of VSMC motility.
Cristina Deflorio (University of Roma La Sapienza): Effect of riluzole on human muscle voltage-gated sodium currents in ALS myotubes.
Michael Di Palma (University of Urbino): Motor activity affects adult skeletal muscle re-innervation acting via Trk receptors.
Pasqua Cancellara (University of Padova): Structure and function of biceps and quadriceps muscles in elderly subjects.
Tatiana Moro (University of Padova), Gender differences in maximal strength improvement and muscle fiber characteristics after 8 weeks of resistance training.
Carlo Bruttini (University of Milano): Hand immobilization affects arm and shoulder postural control.

10.00-10.30 Coffee break

10.30-13.00 **Oral communications:**

Neurophysiology II

Mattia Ferro (Vita-Salute San Raffaele University, Milano): A new method for functional analysis of cerebral circuits.
Diego Ghezzi (The Italian Institute of Technology, Genova): Organic electronics allows the photoelectric excitation of neuronal activity in primary neuronal cultures and acute retinal explants.
Nina Krako (Scuola Normale Superiore, Pisa): Investigating the role of intracellular Alzheimer's amyloid β oligomers in mitochondrial dysfunction through an intrabody approach.
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Simone Sarasso (University of Milano): Local, use-dependent changes in the waking EEG after prolonged wakefulness.

13.00-14.00 Lunch

14.00-15.00 **Round Table: "The future in research: grant opportunities for young investigators"**
Lucia Monaco, Chief Scientific Officer, Fondazione Telethon, Milano; **Jacopo Meldolesi**, Department of Neuroscience, San Raffaele Institute, Milano; **Gabriele Ballero**, Head Projects Office, The Italian Institute of Technology, Genova.
Stories
Flavia Antonucci, Researcher, Department of Medical Pharmacology and CNR Institute of Neuroscience, Milano, Coordinator of a Grant FIRB - The Future in Research, Ministry of Instruction, University and Research; **Vincenzo Lionetti**, Researcher, Scuola Superiore Sant'Anna, Pisa, Coordinator of a Grant for Young Investigators, National Health Service.

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Marie-Therese Noedl (The Italian Institute of Technology, Genova): Characterization of the arm morphology during embryonic development of Octopus vulgaris.
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Claudia Carmone (University of Bari): The role of Store-Operated Cyclic AMP Signalling (SOcAMPS) in cardiac physiology and pathology: an in vitro study on neonatal rat cardiomyocytes.
Daniela Glinni (University of Sannio): Effects of 3,5-diiodo-L-thyronine on skeletal muscle mitochondria from high-fat diet fed rats: a functional/proteomic approach.
Francesco Paonessa (The Italian Institute of Technology, Genova): The transcription factors Sp1 and REST interplay to modulate Synapsin I gene expression.
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Lamberto Maffei, President of the Accademia Nazionale dei Lincei, Roma; Emeritus Professor of Neurobiology at the Scuola Normale Superiore, Pisa

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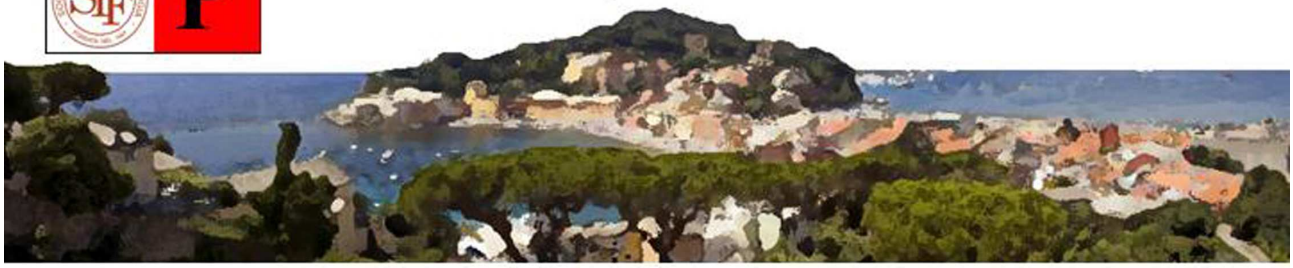
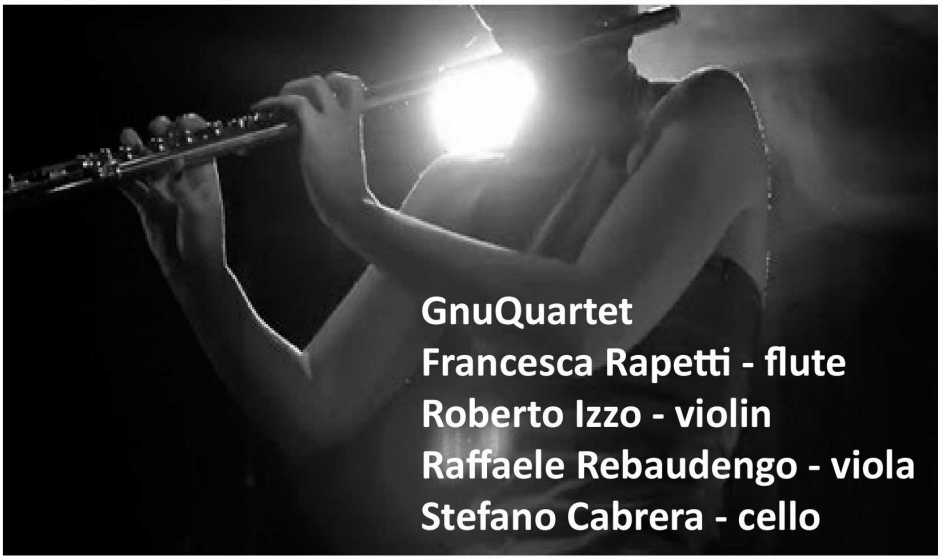
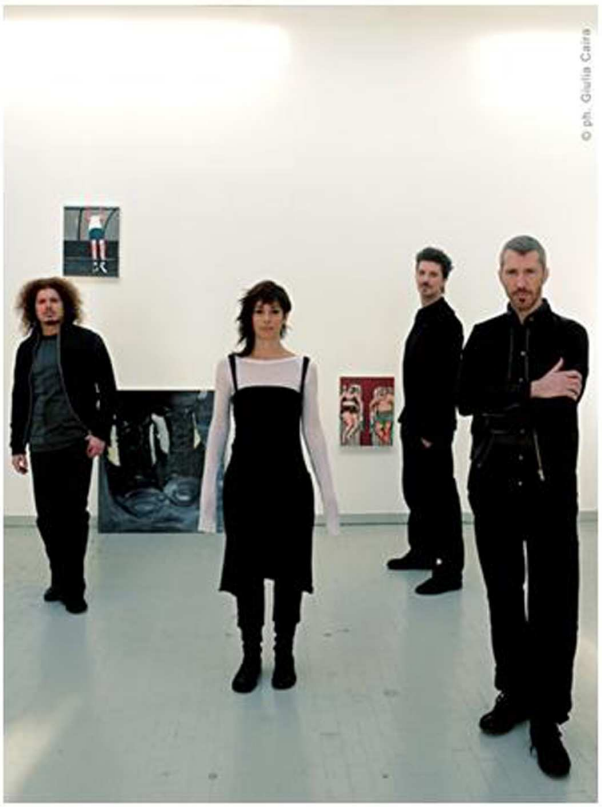




Something Gnu

A concert with new songs and reinterpretations of old songs, that releases the energy of rock, the virtuosity of jazz, the clearness of classical instruments and results in a unique and sophisticated sound. The epic of U2, the touching transparency of Message in a bottle, the sunniness of Mr. T, the humor of Nougaro, the poetry of the classic Italian song "Una giornata uggiosa". The concert is a continuous change of atmosphere and colorful emotions, moving with lightness through r emote influences.



ORAL COMMUNICATIONS

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2) Federica Bertozzi (University of Bologna): Visually guided arm movements in 3D space: spatial tuning in fixation, preparation and reaching activity in monkey medial posterior parietal cortex.
3) Annalisa Bosco (University of Bologna): Multiple reference frames used in encoding reaching activity in the medial posterior parietal area V6A.
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1) Michele Bertacchi
PhD student in Neurobiology (3rd year)
Scuola Normale Superiore, Pisa
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The positional identity of mouse embryonic stem cells-generated neurons is affected by BMP signaling

Michele Bertacchi[1], Luca Pandolfini[1], Elisa Murenu[2], Alessandro Viegi[1], Simona Capsoni[1], Alessandro Cellerino[1], Andrea Messina[2], Simona Casarosa[2], Federico Cremisi[1]

[1]Scuola Normale Superiore, Pisa, Italy
[2]CIBIO, University of Trento, Italy

Central Nervous System (CNS) development is an extraordinarily complex process that requires the orchestrated action of multiple signaling molecules. Classical studies in lower vertebrates indicate that Bone Morphogenetic Proteins (BMPs) inhibit the default differentiation fate of pluripotent embryonic stem cells, which is both neural and anterior. Recent works show that embryonic stem cells (ESCs) generate neurons when deprived of exogenous signals and respond to BMP inhibitors, indicating a role for BMPs also in the neuralization process of mammalian pluripotent cells.
We investigated the impact of BMPs on the positional identity of neurons generated in vitro by mouse ESCs, an aspect that has been neglected so far. To demonstrate which positional identity might emerge during differentiation of ESCs as a default program, we established a method of neurogenesis from ESCs that minimizes the influence of extracellular signals. The method is a three-steps procedure of culture in a chemically defined minimal medium, devoid of serum or morphogens but allowing cell survival by insulin. We characterized ESCs differentiated in minimal medium by the analysis of pan-neuronal markers and of anterior-posterior or dorsal-ventral markers of developing CNS.
We found that ESCs produce, secrete and respond to BMPs during their differentiation in vitro as neurons. Moreover, global gene expression profiles of differentiated ESCs cells were compared to the profiles of embryonic forebrain, midbrain and hindbrain. Differentiated ESCs show a profile consistent with a mixed forebrain-midbrain identity.
We then compared the effects of the BMP inhibitor Noggin with that of Retinoic Acid (RA). Both Noggin and RA support neuronal differentiation of ESCs, but their main effects are on the positional identity of neurons: whereas RA supports the typical gene expression profile of hindbrain neurons, Noggin induces a profile characteristic of telencephalic neurons.
Interestingly, in our experimental system endogenously produced BMPs act on ESCs differentiation mainly by inhibiting the expression of a telencephalic gene expression profile rather than by inhibiting their neuralization.
Our findings show that endogenously produced BMPs affect the positional identity of the neurons that ESCs spontaneously generate when differentiating in vitro in a minimal medium, and support the existence of an intrinsic program of neuronal differentiation with anterior/telencephalic identity.

2) Federica Bertozzi
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Visually guided arm movements in 3D space: spatial tuning in fixation, preparation and reaching activity in monkey medial posterior parietal cortex

Federica Bertozzi, Konstantinos Hadjidimitrakis, Rossella Breveglieri, Annalisa Bosco, Giulia Dal Bo', Patrizia Fattori

Eye position modulation of neural activity has been established as a common mechanism for the localization of visual targets in space in several areas of the posterior parietal cortex (PPC). However, the influence of gaze modulations in subsequent reaching activity has received less attention. Cortical area V6A, located in the medial PPC of primates carries eye position information and contains neurons with arm movement related activity. The aim of our study was to compare the spatial encoding in fixation, preparation and execution of reaches towards foveated targets located at different depths and directions within the peripersonal space.
Single unit activity was recorded from area V6A in one Macaca fascicularis monkey performing a fixation-to-reach task in darkness. Targets were 3D light emitting diodes (LEDs) placed at different positions in the 3D space. In the majority of the cells, a significant effect of both target direction and depth was found in all epochs. Spatial modulations of fixation activity were generally maintained across subsequent epochs and this occurred more frequently in the depth domain. Spatial encoding was remarkably consistent across epochs with common preferences uniformly distributed in 3D space. Present data clearly demonstrate that in an important fraction of V6A neurons eye position modulations can reliably predict the spatial tuning of reach related activity. In addition, they highlight the role of V6A in the processing of eye position signals in order to jointly encode spatial location and hand movement information.

Funded by: FP7-ICT-217077-EYESHOTS, Fondazione del Monte di Bologna e Ravenna (Italy), MIUR.

3) Annalisa Bosco
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University of Bologna
annalisa.bosco2@unibo.it

Multiple reference frames used in encoding reaching activity in the medial posterior parietal area V6A

Annalisa Bosco, Rossella Breveglieri, Claudio Galletti, Patrizia Fattori

Department of Human and General Physiology, University of Bologna, Italy

Arm movement and gaze direction modulate the activity in V6A, a visuomotor area of the macaque medial posterior parietal cortex. In a previous study, it was demonstrated that gaze direction influences the reaching responses of V6A (Marzocchi et al., 2008). In the present work, we analyzed the effect of arm-movement direction on V6A neuronal activity while the eyes were fixating in a constant straight-ahead position. Arm direction influenced reach-related activity in the majority of neurons during the execution of reaching movements (65%) and during static arm postures (59%). We investigated whether the spatial tuning of reach-related discharges was similar or different in the 2 subsectors of area V6A (V6Ad and V6Av) recently identified according to cytoarchitectonic criteria (Luppino et al., 2005; Gamberini et al., 2011). Results show that the spatial preferences of reaching activity and that related with the static arm posture were similar in V6Ad, without showing any significant over-representation of parts of the working space (chi2 test, n.s.). In V6Av, in contrast, reaching activity showed a stronger and statistically significant preference for the ipsilateral space with respect to the central- (foveated) and contralateral space (chi2 test, $P < 0.05$). Both subsectors presented a higher number of neurons preferred reaches directed toward peripheral, rather than foveal targets. Comparison of single cell responses for reaching movements executed in different combinations of eye- and target- configurations showed that V6A neurons code reaching actions according to distinct reference frames. Some neurons coded reaching actions depending on the position of the target in space (spatial cells), others were based on the position of the target relative to the eye (eye-centered cells) and the majority (about 70%) contained both these representations (mixed cells). These data suggest that V6A neurons represent spatial information in a heterogeneous manner and are consistent with the view that V6A is involved in providing spatial sensory information to brain areas generating motor responses.

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The tyrosine phosphatase STEP modulates synaptic function at the presynapse

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STEP (striatal-enriched protein tyrosine phosphatase) is a brain-specific tyrosine phosphatase involved in neuronal signal transduction. STEP opposes the development of synaptic strengthening by dephosphorylating its substrates, which include proteins involved in synaptic plasticity and intracellular signaling. STEP dephosphorylates and inactivates the key signaling molecules MAP kinase ERK1/2 and p38, as well as the tyrosine kinase Fyn, and induce internalization of the ionotropic and metabotropic glutamate receptors (NMDARs and AMPARs). For these reasons STEP opposes long-term potentiation and facilitates long-term depression. Moreover dysregulation of STEP activity contributes to the pathophysiology of several neuropsychiatric disorders with cognitive impairments, including Alzheimer's disease, Huntington's disease, schizophrenia and fragile X syndrome. Although STEP has a predominant role at postsynaptic level, it is also present presynaptically, and may play some role in the regulation of transmitter release. To better understand STEP function at the presynapse we used STEP knockout (KO) mice. STEP KO mice showed an increased glutamate release from synaptosomes after depolarization compared to wild type (WT) and an enhanced phosphorylation of ERK1/2, whose activity was significantly increased also under basal conditions. ERK1/2 phosphorylates synapsin I, a major neuronal phosphoprotein associated with synaptic vesicles and involved in regulation of neurotransmitter release, synapse formation and neurite outgrowth. The phosphorylation state of synapsin I at distinct sites was also changed in STEP KO mice, leading to an increased availability of synaptic vesicles. Besides, from the morphological point of view, we observed an increase in axonal elongation in neurons from STEP KO mice at early stages of development, compared to developing WT neurons. We are investigating further the presynaptic changes associated with STEP deletion to understand how STEP modulates presynaptic function.

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Redox unbalance and metabolic impairment in skeletal muscle of hindlimb unloaded mice: a time-course study

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Introduction: In order to understand the mechanisms underlying disuse atrophy we studied the adaptation of slow and fast muscles (soleus and gastrocnemius) at different time (3 and 7 days) to hindlimb unloading (HU) in mice.
Methods: at 3 and 7 days of HU, we studied : a) redox imbalance through the expression of Nrf2 (the master transcriptional regulator of antioxidant genes), of antioxidant defense systems (SOD1, Catalase) and of protein carbonylation; b) master controllers of the balance between muscle protein synthesis (MPS) and breakdown (MPB) (AKT and p70/S6K; MuRF-1, atrogin-1); c) autophagy (Beclin-1, p62); d) energy metabolism (PGC-1alpha and key metabolic enzymes). To clarify the link between ROS and cell signaling pathways, we administered the antioxidant Trolox during the early phase of disuse atrophy (3 days HU).
Results: Unloading: Gastrocnemius showed fiber atrophy (18%) at 3 and 7 days HU. The ubiquitin-proteasome system (MuRF-1, atrogin-1) was up-regulated and the autophagy system (p62, Beclin1) was activated at 3 days HU only. Furthermore, Nrf2 was up-regulated at 3 days HU, SOD1, Catalase and Hsp70 protein content was higher at 3 and 7 days HU and no protein carbonylation was observed.
Soleus showed fast fibers atrophy at 3 (6%) and at 7 days (14%), whereas slow fibers atrophy was found at 7 days (9%) only. As in Gas, MuRF-1, atrogin-1 and p62 were over-expressed and Nrf2 was up-regulated at 3 days HU only. SOD1 and catalase showed a peculiar expression trend; the first was up-regulated at both times whereas catalase only at 3 days HU. In addition, down-regulation of PGC1alpha and oxidative metabolism enzymes was found at 3 and 7 days HU.
Antioxidant treatment: In Soleus, Trolox prevented the upregulation of Nrf2 and antioxidant enzymes, but not that of Murf1 and atrogin-1; whereas in Gastrocnemius antioxidant treatment does not prevent SOD1, catalase, Murf-1 and atrogin-1 upregulation
Discussion: Unloading data suggest a ROS induced activation of MPB in both Soleus and Gastrocnemius. Furthermore, in Soleus, alterations in PGC-1alpha expression and oxidative metabolism could further increase ROS production and contribute itself to worsen atrophy. In addition, in Soleus, antioxidant treatment seemingly counteracted redox imbalance, but did not prevent degradation pathways activation suggesting that oxidative stress does not play a major role in determining atrophy. In Gastrocnemius, based on the effects of antioxidant treatment, it is not excluded a redox unbalance impact in triggering atrophy. The latter results in Gastrocnemius prompted an ongoing analysis of ROS dose response curve in C2C12 cultures, which will be presented at the meeting.

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The role of Store-Operated Cyclic AMP Signalling (SOcAMPS) in cardiac physiology and pathology: an in vitro study on neonatal rat cardiomyocytes

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Background and Aims: Store-Operated Cyclic AMP Signaling (SOcAMPS) represents a recently identified mechanism of cross-talk between Ca^{2+} and cAMP signals. In this process, depletion of Ca^{2+} in the endoplasmic reticulum (ER) leads to increases in cAMP levels, independently of cytosolic Ca^{2+} changes. Expression and functionality of STIM1 (Stromal Interaction Molecule 1), a transmembrane ER Ca^{2+} sensor protein, is necessary for SOcAMPS to occur. Interestingly, recent reports have demonstrated a critical role for STIM1 in the development of cardiac hypertrophy, a process notoriously controlled both by Ca^{2+} and cAMP signaling. Here we aimed to evaluate whether SOcAMPS was manifest in neonatal rat cardiomyocytes and its potential role in cardiac cell hypertrophy.
Methods: To monitor changes in cAMP levels, real time imaging experiments were performed on neonatal rat cardiomyocytes transiently transfected with an EPAC-based fluorescent probe for [cAMP], EPAC H30. Fura-2 and Fluo-4 were used to monitor cytosolic Ca^{2+} levels and an ER/SR targeted probe, D1ERcameleon, was used to measure ER [Ca2+]. Long term incubation (48h) of cardiomyocytes with angiotensin II (1 μM) and aldosterone (1 μM) was used to induce "in vitro" cell hypertrophy. Increases in cell size and/or sarcomere alignment were monitored microscopically after labeling with phalloidin-TRITC.
Results: To verify the existence of SOcAMPS in neonatal rat cardiomyocytes, cells were stimulated in Ca2+-free Ringer's solutions with the low affinity membrane permeant Ca2+ chelator TPEN (1mM), able to induce a reduction of SR Ca2+ levels ([Ca2+]SR) without affecting cytosolic [Ca2+]. SR Ca2+ measurements demonstrated that under these experimental conditions, 1 mM TPEN led to a reduction in intraluminal [Ca2+] that was 50,5±2,4% (8 exp, 11 cells, $p<0.001$) of the maximal store depletion. Parallel experiments performed with the EPAC H30 cAMP sensor showed increases in [cAMP] that were 26,5±3% (13 exp, 13 cells, $p<0.001$) of the maximum delta ratio. In the presence of 5 μM Forskolin (FRSK) the TPEN-induced cAMP augmentation resulted 63,7±3,9% of the maximal response (16 exp, 19 cells, $p<0.001$). Also depletion of SR by the Ca2+ ionophore ionomycin (10 μM) was found to induce significant cAMP increases both in the absence and presence of FRSK. The participation of STIM1 in the observed phenomenon was proven by the 47 % reduction of the TPEN+FRSK induced [cAMP] signal after transfection of cells with a shRNA against STIM1 (6 exp, $p<0,01$). To evaluate the putative role of SOcAMPS in cardiac hypertrophy, cAMP measurements were performed on angio+aldo treated cells and compared to control cardiomyocytes. Under these experimental conditions a 20% increase of the TPEN+FRSK induced response was observed in hypertrophic myocytes (16 exp, $p<0,01$).
Conclusions: These data straightforwardly establish, for the first time, the existence of SOcAMPS in the neonatal cardiomyocyte cell model. Also, a significantly increased SOcAMP signalling was shown to exist in hypertrophic cardiomyocytes. Further experiments to ascertain whether a cause-and-effect relationship exists between SOcAMPS and cardiac cell hypertrophy are in progress.

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Brain – derived neurotrophic factor (BDNF) synaptic function and dysfunction: focus on beta amyloid toxicity

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Neurotrophic factors of NGF family are critical molecules that support development, differentiation and neuronal plasticity of neurons. In particular, BDNF and its receptors are widely expressed in brain areas exhibiting high degree of neuronal plasticity such as the entorhinal cortex (EC) and hippocampus that are involved in learning and memory. In this study we investigated BDNF effect on synaptic function in the EC. Moreover, since BDNF expression was found to be reduced in EC of patients with Alzheimer disease (AD) and the beta-amyloid (Abeta) has a critical role in the pathogenesis of AD we evaluated BDNF protective role in Abeta-dependent synaptic dysfunction. Synaptic function was studied recording extracellular field potentials (FPs) evoked in cortical layers II–III of EC slices; LTP was elicited by high frequency stimulation (HFS). We evaluated the effects of BDNF perfusing EC slices at different concentrations (40, 20, 4 and 1 ng/mL). We found that at the lowest concentration (1 ng/mL) BDNF does not interfere with basal synaptic transmission leaving normal LTP induction and maintenance. At higher concentrations (40 and 20 ng/mL) BDNF induced a depression of FPs amplitude and reduced LTP magnitude. Thus, BDNF changes synaptic efficacy and strength in EC depending on the ligand concentrations. Next step was to investigate whether BDNF has a neuroprotective role against Abeta toxicity. To this aim different concentrations of BDNF (10-50 ng/mL) were first tested to prevent Abeta neurotoxicity in mouse primary cortical cell culture. We found that Abeta42 induced activation of stress-related kinases (p38, JNK) was significantly reduced by pretreatment with BDNF in the range of nanograms/mL. Using BDNF at 1 ng/mL we were able to completely rescue LTP in Abeta perfused slices. BDNF – mediated protection was mediated by TrkB receptor activation as the effect was completely inhibited by perfusion with K252a (tyrosine kinase inhibitor). The hypothesis can be advanced that BDNF and its receptors represent an endogenous reserve whose change may exert neuroprotection or neurodegeneration.

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Synapsin II mutations associated with autism or epilepsy: effects on neuronal development and synaptic vesicle cycling

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Synapsins (Syns) are a family of neuron-specific phosphoproteins that dynamically associate with synaptic vesicles (SV) and the actin cytoskeleton, regulating neuronal development and synaptic function. Syn knock-out (KO) mice exhibit spontaneous epileptic seizures and mutations in SYN1 gene have been found in humans affected by epilepsy or autism. We here describe a total of four mutations, one frameshift (FSM-1) and three missense mutations (MSM-1,-2,-3), identified in the SYN2 gene in autistic or epileptic patients and investigate on their pathogenic mechanisms.
We analyzed the effects of the mutations on in vitro neuronal development. Cultured hippocampal neurons from SynII KO mice display impaired axonal outgrowth and dendritic arborization compared to wild type (WT) neurons. Transfection of WT, MSM-1 and MSM-2 SynII variants into the SynII KO background rescued the impairment in both axon elongation and dendritic branching. In contrast, neurons overexpressing MSM-3 showed a defect in axon outgrowth and dendritic arborization similar to mock-transfected KO neurons. The FSM-1 mutation severely impacts on cell survival, probably because of its precocious truncation and abnormal primary structure.
We next analyzed the effect of the MSM-1 and MSM-2 variants, having no effect on neuronal development, on SV cycling by live cell imaging experiments. We first checked the ability of the SynII variants to be targeted at the synaptic terminal and revealed expression levels at synapses comparable with WT-SynII. SynII KO neurons presented a significant decrease of reserve pool of SV compared to controls. While expression of WT-SynII in SynII KO terminals lead to a complete recovery of the reserve pool, both MSM-1 and MSM-2 variants failed to rescue the SynII KO phenotype.
The characterization of the molecular mechanisms underlying the synaptic defects caused by SynII mutations will provide novel advances in the understanding of the abnormalities of synaptic structure and function related to epilepsy and autism.

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TBC1D24: a novel epilepsy gene involved in cortical network development

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Epilepsy is a common neurological disorder in paediatric age, affecting 0,7% of children. Thus, the socio-economical burden of epileptic disorders is considerable. Rare monogenic epilepsies represent a unique tool to explore the neurobiological events leading hyperexcitability and seizure, as molecular pathway can be identified and tested in different experimental conditions in vitro and in vivo. We and other demonstrated that TBC1D24, a novel gene, is involved in recessive forms of early-onset idiopathic epilepsy and epileptic encephalopathies. TBC1D24, is mainly expressed in cerebral cortex and hippocampus and encodes a novel ARF6-interacting protein involved in neurite outgrowth.
To get insight into the role of TBC1D24 in cortical development, we used the in utero RNA interference approach, to knockdown TBC1D24 expression in rat embryonic neuronal progenitor cells. Using two different shRNAs, we knocked-down TBC1D24 expression in rat brains at embryonic day 15.5 and showed a delay in radial migration of TBC1D24-knockdown neurons into the cortical plate at embryonic day 20, with most of cells showing morpho-functional abnormalities. We also showed that this abnormal migration pattern is not due to defects in the neuronal differentiation.
Moreover, concomitant expression of wild-type TBC1D24 and shRNA prevented migration delay, whereas pathogenic variants failed to complement RNAi effect, suggesting a loss-of-function mechanism in patients. In contrast, we previously showed that the TBC1D24 overexpression resulted in a significant increase in neurite arborisation whereas pathogenic variants reverted this phenotype.
However, analysis at postnatal stage of development revealed that TBC1D24-knockdown neurons reached appropriate cortical layers, although featuring significant defects in morphological maturation. We intend to assess ARF6 involvement in the TBC1D24 mediated cortical developmental processes. In conclusion, our data revealed a developmentally regulated role of TBC1D24 in cerebral cortex formation suggesting that mutations in TBC1D24 may cause subtle alterations in cortical development leading to epilepsy.

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A new method for functional analysis of cerebral circuits

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An important avenue in neuroscience is represented by a more in depth analysis of cortical activity, the expectation being to find novel correlations between specific animal behaviours or cognitive functions and unique patterns of activity in neurons and synaptic networks. This goal can be reached thanks to the development of novel methodologies that ideally should be sensitive enough to provide quantitative information about single elements but also providing a view of the activity in the entire cortical network. In my laboratory in the last few years we have developed a series of biosensors for the investigation of synaptic activity both in vitro and in the animal in vivo. In order to develop a genetically encoded indicator of synaptic network activity, we have generated a series of reporters of synaptic vesicle re-use. These sensors have been named as the GreenZip family. These indicators report synaptic activation through the uptake of small fluorescent peptidic markers during cycles of exo-endocytosis, whose frequency is greatly enhanced by synaptic transmission and neuro-transmitter release. These new tools have been engineered by modifying the scaffold of the vesicular protein VAMP2 (Synaptobrevin2) through the insertion, at the intraluminal ending, of a "bait" domain with binding activity for a 4 kD peptide dubbed Synbond. The latter is conjugated with a fluorophore or with other detectable molecules. This pair of binders was selected for their high binding affinity (in the nM range) and the reporter gene was named GreenZip (the prefix Green indicate the presence of a GFP molecule at the N-terminal, cytosolic domain). These constructs have been shown to work in cultured neuronal networks (dissociated cultures of hippocampal neurons). When these constructs are expressed in neuronal cells, the sensor is correctly inserted into synaptic vesicles which are then sorted to synapses. The activity-dependent uptake of Synbond was characterized in detail and found to correlate well with synaptic efficacy and with the frequency of stimulation of presynaptic cells. To test the feasibility of this method for in vivo analysis, this family of molecules was expressed by electroporation of cDNA in brain slices (cortical, cerebellar and hippocampal cultured slices) and in vivo in the LGN thalamic nuclei (by cDNA electroporation in retinal ganglion cells). In these experiments, we demonstrated that Synbond, diffuses quickly across brain tissue and reaches synapses. Therefore, this technique permits unprecedented in vivo recordings from large synaptic networks with very high spatial and temporal resolution. These experiments were run in living animals and the detection of GreenZip-expressing synapses (by GFP) and of Synbond uptake was obtained retrospectively after sacrificing the animal, because the thalamus is located too deeply inside the brain to be reached by available optical technologies. To be able to express GreenZip molecules at the brain surface in neocortical areas, we have developed a transgenic model capable of expressing GreenZip potentially in every tissue and at any time point in development (the Rosa26Greenzip mouse line). In parallel we are generating a family of lentiviral vectors to express GreenZip and its molecular variants more selectively using stereotaxic injections of the viral vectors inside specific subgroups of cortical cells. At the meeting I will present my contribution to this work.

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Characterization of the Octopus arm morphology and its regenerative capability: role of Acetylcholinesterase during morphological modification

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The ability of regenerate whole-body structures has been long studied and still has a peculiar interest in stem cell biology for its therapeutic potential. A regenerative process implies the renewal, restoration, and growth of cells, tissues and organs that have been physically or functionally lost. Regeneration of arms in Cephalopods and in particular in Octopods has been the subject of several studies.
The first study on the regeneration of the Octopus arm demonstrated that all regenerated tissues, with the exception of the dermal connective tissue, are produced by the pre-existing tissues of the same kind. However, the details of the regenerative process in the Octopus arm remain to be elucidated. Using Octopus vulgaris as model of regeneration, we are investigating the role of different cell types and neurotransmitters in the regenerative process. Since Octopus has a typical cholinergic innervation at the level of the arm neuromuscular system, we investigated the involvement of acetylcholinesterase (AChE) in the Octopus arm regeneration. AChE has been demonstrated to have non-cholinergic functions in various cell types and to be involved in the regulation of cell proliferation, differentiation and apoptosis. Our hypothesis is based on previous studies, which showed that in lower invertebrates and amphibians different expression levels of AChE are associated with the regeneration phases. In order to follow cell replacement in the Octopus arm, we first assessed the expression of specific markers involved in cellular proliferation (AgNOR and PCNA). Our results showed that the activity of the enzyme AChE is related to the proliferation stage of the arm regenerative process. In the very initial stages of regrowth when most of the proliferation activity was at the level of the 'blastema' the cholinesterase activity was very low. AChE activity climbed slowly during the subsequent phase of cellular multiplication and, by the onset of morphogenesis, the activity rose sharply and active myogenesis was observed. AChE activity decreased then till reaching basal level at the time when the process of histogenesis occurred and the reestablishment of all the structures became evident. Interestingly AgNOR and AChE assay showed a similar trend in particular during the stages when the morphogenesis was mostly dependent upon cell proliferation. We suggest that AChE protein may have an important influence in the process of regeneration and that it could be considered as a potential target to promote or regulate the regenerative process. In order to follow the expression of the AChE gene during regeneration we are now completing the cloning of the entire gene of AChE. AChE is a molecule that evolved very early in evolution and is well conserved across species. So far we obtained a fragment of 1044 bp, corresponding to a protein of 348 aa, which has high homology with the AChEs of other animal species. Our next step is to produce a probe for in-situ experiments to detect different levels of expression of AChE in samples at various stages of regeneration. This will allow us understanding some latent basic pathways to unlock to promote regeneration.

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Organic electronics allows the photo-electric excitation of neuronal activity in primary neuronal cultures and acute retinal explants

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Interfacing organic electronics and biology offers new possibilities in biotechnology, due to the unique properties exhibited by organic conducting polymers (e.g. biological affinity, mechanical flexibility, ease of functionalization and cost effectiveness). Organic conducting polymers have been exploited as materials for cellular interfaces in several fashions as: (i) passive electrode coatings or culturing substrates, (ii) organic biosensors or (iii) actuators for neurotransmitter release and electrodes for controlled cell seeding, growth and activity detection. Very recently, an organic photovoltaic donor-acceptor blend has been exploited for neuron stimulation by a photo-electric process. With respect to previous examples with inorganic semiconductors, this system has several advantages including flexibility, no power requirement and biocompatibility. Here, we report the novel use of a single component semiconductor organic polymer for the direct control of neuronal activity. This interface, that is more efficient than the classical bulk hetero-junction interface, has the remarkable capability to evoke excitation of neuronal firing in response to illumination. We demonstrate that the polymer layer has the ability to induce action potential firing up to 20 Hz in cultured hippocampal neurons. Moreover, this interface has been exploited to restore visual response in retinal explants obtained from animal models of retinal degeneration (light-blinded albino SD rats). By recording local field potentials in the RGC layer, we demonstrated the ability of the organic conductive polymer to mimic the function of photoreceptors and induce retinal activation of retinal ganglion cells after light illumination. These results paved the way to the development of a new and disruptive technology for interfacing artificial devices with neuronal networks, with applications in neuroprosthesis and brain machine interface research.

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Pentylenetetrazole-induced epileptiform activity impairs basal synaptic transmission and short-term plasticity in monosynaptic connections

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Epilepsy, a significant neurological disorder affecting 0.5-1% of the population, is characterized by abnormal electrical discharges in the brain and seizures. Despite high prevalence and extensive research, the mechanisms underlying both onset and progression of epilepsy still remain unknown.
Generally, epileptic activity is very often induced by local application of epileptogenic drugs, including pentylenetetrazole (PTZ), largely employed in both vertebrate and invertebrate neurons. The buccal ganglia neurons of the land snail, genus Helix, have been widely used to study epileptiform activity from an electrophysiological and pharmacological point of view, since the PTZ-evoked paroxysmal activity resembles those described in mammalian neurons in numerous respects. Nevertheless, neuronal networks in intact ganglia have been analyzed so far, not considering the possibility to have a well-defined monosynaptic connection to study convulsant-induced molecular changes without unspecific effects due to the surrounding tissue.
Here, we examined PTZ-induced neuronal changes on Helix monosynaptic circuits formed in vitro, as a simpler experimental model to investigate the cellular and molecular mechanisms induced by epileptic-like activity.
To this aim, we first treated hyperpolarized B2 neurons in synaptically isolated condition to determine their reliability as postsynaptic cells during our experiments. We found that convulsant doses were not sufficient to overshoot hyperpolarizing current injection and elicit action potential firing. Moreover, no alteration in either input resistance or the evoked response to neurotransmitter application has been observed, thus allowing us to investigate presynaptic differences independently from postsynaptic responsiveness.
On the basis of these results, we analysed the effect of epileptiform activity on either basal release or post-tetanic potentiation (PTP), a form of short-term plasticity, recording synaptic connections immediately after the washout and several minutes later. Only at 15 and 30 minutes after PTZ washout, we observed a significant increase of the first evoked postsynaptic potential followed by a robust synaptic depression. In addition, we found that PTP is impaired in all the experimental times following the treatment. Taken together, these data suggest an intermediate-lasting impairment in the dynamic reorganization of synaptic vesicle (SV) pools that follows the prolonged stimulation of synaptic transmission during epileptiform activity.
In order to explain this imbalance, we assessed whether epileptic activity is related to synapsin phosphorylation level, since these proteins are well known to modulate synaptic plasticity tethering SVs to actin cytoskeleton. Using phosphospecific antibodies, Western blot and immunocytochemical staining displayed a PTZ-dependent strong increase of synapsin phosphorylation in both PKA/CaMKI/IV and MAPK sites, two kinase pathways involved in regulating synaptic plasticity.
Our findings show that prolonged epileptiform activity leads to an increase of the synapsin phosphorylation state, virtually losing SV pool segregation, thus determining an impairment of

synaptic strength in both basal condition and tetanus-induced potentiation. Through this mechanism, the long-lasting firing activity and the repetitive phosphorylation events may induce a protracted alteration of synaptic transmission which could dramatically increase the severity of epileptic seizures.

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Effects of 3,5-diiodo-L-thyronine on skeletal muscle mitochondria from high-fat diet fed rats: a functional/proteomic approach

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Impaired insulin action in skeletal muscle (SKM), the bulk of whole-body insulin-mediated glucose uptake, is integral to the clinical manifestations of type 2 diabetes and insulin resistance (IR). In situations of chronic fat overload, SKM is faced with increasing amount of lipids which, if not completely oxidized, might accumulate thus inducing derangements in insulin signaling and holding IR. Evidence has been provided that IR may be accompanied by mitochondrial dysfunction, which could be the proximal cause of impaired lipid oxidation and increased accumulation of intramyocellular lipids (IMCL). Therefore, interventions that improve mitochondrial function have the potential to improve IR. Although a number of therapeutic options are currently available for the treatment of metabolic dysregulations, due to their additional unwanted side effects, the development of safe and effective drugs is a major priority. We have shown that a natural thyroid hormone derivative, the 3,5-diiodo-L-thyronine (T2), when administered to high fat diet (HFD)-fed rats, leads to: a) reductions in body weight and fat-mass gain, b) increases in hepatic fatty acid oxidation, and c) improved metabolic parameters, without causing thyrotoxicosis. In addition, T2 improves glucose tolerance by preventing IMCL accumulation and IR, with a sparing of lean mass and an increase in fast/glycolytic muscle fibres. However, the response of muscular cell metabolism to T2 treatment is unknown. Here, we investigated the in vivo effects of 4-weeks administration of T2 to HFD rats on the skeletal muscle mitochondrial phenotype with a particular focus on mitochondrial protein profile and function by means of two-dimensional gel electrophoreses (2D-E), nLC–ESI-MS/MS and Blue-Native (BN) PAGE. T2, in a context of reduced systemic lipid accumulation, produces muscle specific effects leading to a significant modulation of mitochondrial soluble proteome. Importantly, enzymes involved in mitochondrial oxidative metabolism, among which carbonic anhydrase III, and ATP synthase subunit alpha, were down-regulated in muscle from HFD-T2 rats (vs HFD ones). These data support that T2 leads, in skeletal muscle, to a structural and metabolic shift towards a glycolytic phenotype. Moreover, Ingenuity Pathways Analysis (IPA) identified, among the most enriched functions and pathways affected by T2, energy production/carbohydrate metabolism and oxidative phosphorylation/glycolysis, respectively. Network analysis of the interrelation between differentially expressed proteins identified glyceraldehyde-3P-dehydrogenase (GAPDH) and tumor necrosis factor alpha (TNFα) as the highest-scoring nodes involved in the effects of T2 on muscle mitochondrial metabolism. Furthermore, BN-PAGE based analysis of respiratory chain complexes revealed that T2 significantly modulates the activity of complex I, V and IV and enhances heavier respiratory supercomplexes suggesting a functional mechanism that is directed towards advantaging the muscle respirosome in HFD-T2 rats. Accordingly, polarographic analyses of mitochondrial respiration rates showed that T2-treatment induced a more efficient utilization of carbohydrate substrates.
In conclusion, the approaches used throughout this study and the obtained results provide additional insights into the mechanisms elicited by T2 underlying the fuel-switching capacity of SKM and its respirosome organization, both of which are important for muscle's ability to face with IR associated with fat overload.

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A 'multimenu' system based on Brain Computer Interface for writing with the P300 component of the EEG

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Brain-computer interfaces (BCI) are human-to-machine information channels which produce external actions by using brain signals of voluntary commands independent from muscular output. They are a promising technique to give independence and improve quality of life to patients with severe lesions of the central nervous system. When the motor lesion is particularly severe, writing and speaking are precluded and BCI systems become an important tool to allow communication.
In the present study, an electroencephalography (EEG) based BCI spelling system is proposed, which uses a 'Multimenu' selection matrix (3 x 3) of entire words instead of single letters, and a semantic tree-shape organization.
Seven healthy subjects participated to the experiments. The P300 component of the EEG was used. Each subject underwent two experiments in two separate days. In the first experiment, performance of the subjects in the 'Multimenu' system was tested by using a total of 60 choices (30 'externally-imposed' selections and 30 'free-choice' selections). The accuracy was high in all the subjects with no differences between the methods of selection (average of all the subjects equal to 87%). This result suggested that the P300 component was properly detected, allowing to proceed with the second experiment. Here, 3 x 3 matrices were compared with 6 x 6 matrices. Each matrix type was composed by letters or words, for a total of four matrices. EEG was analyzed by means of the BCI2000 software. Classifier accuracy, speed of selection, bit rate and amplitudes of the evoked P300 were evaluated among conditions.
Results showed that the 3 x 3 'Multimenu' obtained the same level of classifier accuracy as the 6 x 6 matrices, even if its P300 amplitudes resulted significantly lower. We observed that the level of mean accuracy for all the subjects in 3 x 3 matrix with letters was 97%, while mean accuracy for 3 x 3 matrix containing words was 98%. The amount of mean accuracy for both the 6 x 6 matrices (i.e. the one with letters and the one with words) was the same, equal to 99%. However, speed of selection was increased when using the 3 x 3 matrices with respect to the 6 x 6 matrices. We observed that the amount of transferred bits for each selection was higher in the 6 x 6 matrices compared to the 3 x 3 ones.
However, the data about bit rate (amount of transferred bits/one minute) for the different matrix sizes and stimulus types showed an higher bit rate for 3 x 3 matrices with respect to 6 x 6 matrices. These results allow the user to transfer more information in a defined time range using the 3 x 3 matrix with respect to the 6 x 6 matrix.
The evidences coming from the present experiments are in favor of the use of a 'Multimenu' system. Its use is equally effective but faster than the conventional matrices based on single letters. Moreover, it is based on simplified matrices and on words selections that allow a direct access to related submenus, in order to obtain a facilitated and faster communication with respect to more conventional spelling paradigms.

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Investigating the role of intracellular Alzheimer's amyloid β oligomers in mitochondrial dysfunction through an intrabody approach

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The amyloid-β peptide (Aβ) has a central role in Alzheimer's disease (AD). However, the precise mechanism of its role in the pathology is still elusive and the significance of different amyloid-β species has to be clarified. Recently mitochondrial dysfunctions and energy metabolism deficiencies have been linked to the pathogenesis of AD. Although the specific mechanisms leading to mitochondrial failure in AD still remain unknown, a substantial body of evidence indicates that Aβ promotes oxidative stress and dysfunctions of cellular energy metabolism. We study different cellular models of Aβ oligomerization: CHO cells expressing human non mutated amyloid precursor protein (APP) - 7WD4 cells and 7PA2 cells that harbor human APP with mutation (V717F) associated with familial Alzheimer's disease (kindly provided by Dr D. Selkoe). These cells are very well characterized models for production and secretion of the toxic Aβ oligomeric species. While studying the Aβ production inside these cells we are investigating their effects on the cellular and mitochondrial functions and their possible role in Alzheimer's disease (the results on mitochondrial physiology will be presented). In order to investigate the role of Aβ oligomers inside the cells we exploit the intrabody approach (Biocca and Cattaneo, 1995) and the availability of a recombinant anti Aβ conformational intrabody (scFv A13) (Meli, 2009). By targeting scFv A13 to different intracellular compartment we are able to interfere with the formation and secretion of specific Aβ oligomeric species. In order to study the role of Aβ oligomers in the observed mitochondrial dysfunctions, we plan to study mitochondrial physiology in 7PA2 cells that stably express anti Aβ oligomers scFv A13 in the endoplasmic reticulum (7PA2_A13K), in the mitochondria (7PA2_A13Mtet) or in the ER-associated degradation pathway (7PA2_A13D).

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17 β-Estradiol modulation of cell proliferation requires diverse post-translational modification of estrogen receptor α

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The sex hormone 17β-estradiol (E2) exerts its pleiotropic effects through the binding to the ligand-activated transcription factor estrogen receptor alpha (ERα). The E2:ERα complex regulates several physiological processes (e.g., reproductive functions) including the fine control of cell survival and proliferation. The main textbooks of endocrinology dictates that E2 binding to ERα induces receptor nuclear translocation, where ERα directly binds to the estrogen responsive elements (ERE) located in the promoter of the E2-responsive genes, thus regulating gene transcription (i.e., nuclear signalling). This picture has been challenged in recent years with the discovery of E2 ability to also elicit rapid cellular effects that occur in seconds to minutes after hormone engagement and are independent on ERα transcriptional activity. This extra-nuclear signalling involves the activation of a membrane-localized ERα through enzymatic ERα palmitoylation that controls the E2-mediated activation of signalling pathways (e.g., ERK/MAPK; PI3K/AKT), which are necessary and sufficient for the E2-dependent control of cell proliferation and survival. Many post-translational modifications occurs on ERα and are regulated by E2 in the coordination of the E2:ERα-dependent cellular effects (e.g., cell proliferation). Indeed, E2 induces the serine (S) residue 118 phosphorylation that facilitates ERα binding to DNA and gene transcription. Moreover, our research group has previously demonstrated that E2 reduces ERα palmitoylation on the cysteine (C) residue 447, thus modulating the amount of the receptor located at the plasma membrane and the ability of E2 to trigger extra-nuclear signalling that leads to cell proliferation.
The ERα is also an ubiquitinated protein. ERα polyubiquitination (polyUbq) is a mean to regulate its turnover through the 26S proteasome. Indeed, ERα polyUbq increases on E2 binding and ERα degradation occurs. Moreover, ERα monoubiquitination (monoUbq), a non-proteolytic signal involved in a network of several different physiological processes, has been found in vitro and in cell lines. Our work has been aimed to analyze the E2-dependent interplay of ERα ubiquitination with other known receptor post-translational modifications and to clarify its role in the regulation of E2-induced cell proliferation.
Our results demonstrate that ERα polyUbq and monoUbq cross-talk with ERα S118 phosphorylation and palmitoylation and are required for the E2-dependent control of cell proliferation. In particular ERa monoUbq is negatively modulated by E2 and the mutation of the ERα monoUbq sites prevents E2-induced S118 ERα phosphorylation, reduces ERα transcriptional activity, and precludes the ERα-mediated extra-nuclear activation of signaling pathways required for E2-induced cell proliferation. Moreover, we discovered that the lack of ERα palmitoylation fastens E2-induced polyubiquitination-dependent ERα degradation and prevents receptor phosphorylation, thus resulting in a blockade of the E2:ERα-regulated gene transcription. Altogether, these data demonstrate that degradative and non-degradative ERα ubiquitination contribute to E2-mediated cell proliferation.
In conclusion, our study reveals a new model of E2:ERα cellular signalling in which nuclear and extra-nuclear ERα activities represent a unique molecular circuitry that is finely regulated by a code several ERα post-translational modifications and defines a new integrated cellular model which could account for the E2-mediated pleiotropic effects.

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miR-361 cooperates with SRIF to prevent hypoxia-induced accumulation of VEGF in HUVEC

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Introduction: Hypoxia stimulates angiogenesis by inducing the expression of several pro-angiogenic factors, including vascular endothelial growth factor (VEGF). Recent findings indicate that small non-coding RNA molecules, called microRNAs (miRNAs) bind to mRNA targets and affect their translation thus regulating several processes, including angiogenesis. It is known that the expression of miRNAs is tightly controlled, but limited information are available on miRNA function in endothelial cells. In human umbilical vein endothelial cells (HUVEC), we have previously demonstrated that somatostatin (somatotropin release inhibiting factor, SRIF), a widely distributed polypeptide with antiangiogenic activity, prevents hypoxia-induced up-regulation of VEGF through the activity of the hypoxia-inducible factor 1 (HIF-1) and the signal transducer and activator of transcription 3 (STAT3; Dal Monte et al., 2011). Aim of the present study was to evaluate the role of miRNAs in the anti-angiogenic activity of SRIF.
Materials and Methods: We used specific algorithms to identify miRNAs involved in the post-transcriptional modulation of angiogenesis-related genes. miRNA expression was evaluated by realt-time RT-PCR (qRT-PCR) in HUVEC cultured in either normoxic or hypoxic conditions. In addition, we evaluated the role of SRIF on miRNA expression. To explore the possibility that hypoxia-sensitive miRNAs regulated by SRIF may play a role in the SRIF-mediated regulation of angiogenesis-related genes, we transfected hypoxic HUVEC with miRNAs either alone or in combination with SRIF and we determined i. the expression of VEGF, HIF-1α and STAT3 mRNAs by qRT-PCR and ii. VEGF, HIF-1α and pSTAT3 proteins by Western blot. In addition, we used ELISA to evaluate the effect of miRNAs, either alone or in combination with SRIF, on VEGF release.
Results: We identified three novel miRNAs involved in the post-transcriptional modulation of angiogenesis-related genes. We found that hypoxia reduced the expression of two selected miRNAs and that SRIF prevented the hypoxia-induced down-regulation of miR-361, a miRNA targeting VEGF. In addition, we demonstrated that miR-361 did not affect hypoxic levels of HIF-1α and STAT3 while prevented the hypoxia-induced up-regulation of VEGF and cooperated with SRIF in regulating VEGF accumulation in response to hypoxia. In conclusion, our results demonstrate that SRIF may synergize with anti-angiogenic miRNAs in preventing the acquisition of an angiogenic phenotype by endothelial cells.
Conclusions: Our results are the first demonstration that SRIF controls miRNA expression in HUVEC and suggest that miR-361 may participate to the mechanisms underlying the anti-angiogenic activity of SRIF.

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The neural correlates of auditory orienting in macaque monkey: a role for PEEF in species specific vocalization recognition and head motor control

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Hypothesis as for auditory system hierarchical organization suggest a similar organization of visual system. Anatomical and physiological data propose auditory system consisting of two streams, dorsal and ventral, which project together to the frontal lobe. The dorsal one brings auditory spatial information while the ventral one brings sound recognition information. One hypothesis could be that in dorsolateral frontal cortex there is an integration of both auditory streams. Many electrophysiological investigations follow this line of thought. In previous reports, we showed as in "Premotor-Ear-Eye Field" (PEEF), previously called area 8B, there are different kinds of auditory, auditory-motor and motor neurons. For what concerns auditory neurons, we observed, by unit activity recording, that their activity was related to complex environmental auditory stimuli firing for experimenters' voice recognition and sound's spatial direction; on the other hand, auditory-motor neurons activity was related to both auditory complex stimuli and eye and/or ear orienting movements while motor neurons activity was related to orienting ear movements. Several lines of evidence have shown that other regions such as FEF, SEF are involved in the orienting processes and microstimulation and unit activity recording studies show that these areas are involved in eye and ear movements. Moreover, other experiments suggest an involvement of SEF and FEF in the control of the head rotation. In fact, the primates can move their eyes either alone or in coordination with the head. The mechanisms underlying the coordination of these complex movements, called gaze shifts, has been studied extensively with the use of visual targets under various experimental conditions. For all these reasons we investigated a possible role of PEEF in head motor control and in species specific vocalizations.
Here, we show some PEEF's neurons firing for species-specific vocalizations like human's "oou" and monkey's "coo" which confirm previous results; moreover we show some PEEF's neurons related to the head position. These data and our previous investigations support the functional role of PEEF as link between the auditory and motor systems. We propose PEEF as an important node for gaze and attention shift toward auditory stimuli with high motivational significance, constituting a high order region of auditory system.

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Late-onset bursts evoked by mossy fiber bundle stimulation in unipolar brush cells: evidence for the involvement of H- and TRP-currents

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Synaptic transmission at central synapses has usually short latency and graded amplitude, thereby regulating threshold crossing and the probability of action potential generation. In the granular layer of vestibulo-cerebellum, the unipolar brush cells (UBCs) receive a giant synapse generating a stereotyped EPSP-burst complex with early-onset (~ 2 ms) and high reliability. By using patch-clamp recordings in cerebellar slices of the rat vestibulo-cerebellum, we found that mossy fiber bundle stimulation also evoked (in ~85% of cases) a late-onset burst (after tens to hundreds milliseconds) independent from EPSP generation. Different from the early-onset, the late-onset burst delay decreased and its duration increased by raising stimulation intensity or the number of impulses. Though depending on synaptic activity, the late-onset response was insensitive to APV, NBQX and MCPG perfusion and did not therefore depend on conventional glutamatergic transmission mechanisms. The late-onset response was initiated by a slow depolarizing ramp driven by activation of an H-current (sensitive to ZD7288- and Cs^{+}) and of a TRP-current (sensitive to SKF96365), while the HVA and LVA Ca^{2+}-currents (sensitive to nimodipine and mibefradil) played a negligible role. The late-onset burst was occluded by intracellular cAMP. These results indicate that afferent activity can regulate H- and TRP-current gating in UBCs generating synaptically-driven EPSP-independent responses, in which the delay rather than amplitude is graded with the intensity of the input pattern. This modality of synaptic transmission may play an important role for regulating UBC activation and granular layer functions in the vestibulo-cerebellum.

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Schwann cells and mesenchymal stem cells as functional tool promoting peripheral nerve regeneration

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Peripheral nerve regeneration is a remarkable issue which is currently addressed with microsurgery, although it seldom leads to total functional recovery (Chiono V, 2009). Hence nerve bioengineering research focused on the development of bioartificial nerve conduits in order to guide axonal regrowth with the addition of specific cells to speed up this process. Schwann cells (SC) form myelin in the peripheral nerves and play a role in the regenerative process. In experimental studies transplantation of SC improved regeneration due to their ability to release neurotrophic factors, to promote cell adhesion molecules and the extracellular matrix. The ideal transplantable cell should be easily accessible, capable of rapid expansion and integration in the host tissue. However, the use of autologous SC may be impractical due to the technical difficulties in harvesting. Recently there has been considerable interest in the use of adult stem cells as suitable SC alternative (Terenghi et al.,2009). Stem cells are easily accessible and rapidly expandable, and adult stem cells from adipose tissue (ASC) or bone marrow (BM-MSC) can be differentiated into a SC-like (dASC and dMSC respectively) phenotype for nerve injury repair (Tohill et al., 2004). In vitro, glial growth factor stimulated MSC and ASC express S100 and its expression is maintained following in vivo transplantation. Using an in vitro model of co-culture with adult dorsal root ganglia (DRG) neurons, the capacity of the dMSC and dASC to promote axon myelination was verified. Both cell types expressed transcripts for protein zero, peripheral myelin protein-22 and myelin basic protein. Since the potential of stem cells in nerve repair may be limited by innate cellular senescence, it was essential to determine the effects of donor age on morphology and functionality of stem cells. The proliferation rate, the expression of senescence markers (p38 and p53) and the stimulation of neurite outgrowth from DRG neurons were very similar by stem cells isolated from neonatal, young or old rats. Although the distribution and ultrastructure of mitochondria in dMSC and dASC from young and old rats were quite different and seem to indicate physiological senescence of the aged cells, the glial lineage differentiated from aged cells retained the potential to support axon regeneration. Given the wide-ranging influence of Notch signalling in cell differentiation and self-renewal in mammals, its role in the differentiation of stem cells to SC was also investigated. The mRNA for Notch-1 and -2 receptors were expressed in the dASC, and the blockage of notch signalling did not affect the neurotrophic and myelination potential of dASC. Therefore, the differentiation process was independent of the Notch signalling pathway. The potential of stem cells to promote nerve regeneration was also seen in vivo, in fact their transplantation of dMSC into a nerve gap injury model improves regeneration.

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LRRK2 interacts with synaptic vesicles at the presynaptic level

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Parkinson's disease (PD) is a common neurodegenerative disease clinically characterized by bradykinesia, rigidity and resting tremor. Recent studies have enlightened that synaptic dysfunction, implicated in numerous studies of animal model of PD, is both a key factor in PD and an early stage marker in presymptomatic patients. Although the majority of cases are sporadic, mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene are linked to late-onset autosomal dominant PD.
LRRK2 has a molecular weight of approximately 280 KDa and contains several domains including a kinase domain and WD40 domain. LRRK2 function is still far from being elucidated as well as its role in the pathogenesis of PD. At this regard, recent studies have shown that the most common disease segregating mutation G2019S in the kinase domain increases LRRK2 kinase activity, whereas the G2385R mutation associated with increased risk in PD in Asian population may affect WD40 domain function.
In order to characterize the role of LRRK2 and its mutations we have first analyzed the subcellular distribution of LRRK2 and demonstrated that LRRK2 is present in the nerve terminal and in the fraction containing pure synaptic vesicle (SV).
Than to characterize the role of WD40 in the interactions of LRRK2 with SV we have analyzed the interactions between WD 40 domain WT and mutated (expressed as fusion protein with GST) with native SV and SV depleted by proteins associated (SSV obtained through salt treatment).
Our data suggest that LRRK2 tethers SV through its WD40 domain: specifically using WT WD40 domain in binding experiments, we showed that this domain is able to efficiently bind SV and that salt treatment does not reduce this association. Conversely, mutant WD40 retains the ability of binding SV but this binding is significantly reduced in the SSV.
In order to establish if and how LRRK2 kinase activity modifies its binding to SV, we quantified the amount of LRRK2 in SV, in presence or absence of a selective inhibitor of the kinase activity: the result shows that the reduction of the kinase activity corresponds to a reduction of the binding.

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Ascorbate/epigallocatechin-3-gallate/gemcitabine combined treatment kills mesothelioma via DAPK2-dependent noninflammatory apoptosis

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Malignant mesothelioma (MMe) is an asbestos-related, poor-prognosis cancer arising from mesothelial cells and lacking a satisfactory therapy. We had previously shown that ascorbate is synergistically cytotoxic to MMe cells with either gemcitabine or epigallocatechin-3-gallate (EGCG). In this study, by using Chou and Talalay's combination index, we showed that the triple combination ascorbate/EGCG/gemcitabine is more synergistic in vitro than any binary combination of the three compounds. We also tested this combination in vivo, using an ip xenograft model of MMe in immunodeficient mice. Treatments by ip injections resulted in higher survival of mice, as shown by Kaplan-Meyer curves. Necropsy data revealed marked reduction of tumor growth, complete inhibition of diaphragm metastatization, and disappearance of abdominal hemorrhage. Immunohistochemical analyses of tumor tissue showed a reduction of cell proliferation and an increase of apoptosis rate in treated mice, while multiplex analysis for protein phosphorylation indicated a repression of cell growth pathways. Such a complex of data suggests that the triple mixture may act through a synergistic induction of apoptosis. We therefore explored more in detail the mechanism of action on in vitro proliferation of MMe cells. The degree of apoptosis induction was explored by using single compounds at previously-determined IC50 concentrations, or the triple mixture with each compound at (IC50)/3, yielding equipotency under the hypothesis of additivity. Confocal calcium imaging and caspase 3 assay revealed higher increases of these parameters after exposure to the triple combination with respect to any compound used singularly, thus confirming a synergistic induction of apoptosis. Thereafter, by using the Human Apoptosis qPCR StellArray™ technology we made a survey of 96 genes involved in programmed cell death. After statistical comparison of fold changes between treated and untreated cells, the Death-Associated Protein Kinase 2 (DAPK2) gene resulted maximally upregulated, showing a strong synergistic activation. Strong up-regulation of TNSFR11B, coding for osteoprotegerin (OPG), and downregulation of tumor necrosis factor-α-induced protein 3 (TNFAIP3), argued for a repression of NF-κB activity, which was confirmed by western immunoblot analysis of the phosphorylated p-65 NF-κB subunit. Other main gene variations included downregulation of BAG4, BCL2, and XIAP genes, consistent with apoptosis induction, and upregulation of SOD, LTA (lymphotoxin-α), HSPA1A (heat shock protein), and AKT1, pointing to cellular response to stress. Also, downregulation of VEGFA indicated an antiangiogenic effect, thus being in line with antitumor activity. In conclusion, we have demonstrated that the combination of ascorbate/EGCG/gemcitabine synergistically inhibits MMe growth through the induction of noninflammatory, DAPK2-dependent apoptosis. These data indicate our combined treatment as a possible candidate for a novel, innovative clinical approach to mesothelioma.

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Ebf genes regulate myelin formation in vitro

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Ebf genes are a family of helix-loop-helix transcription factors highly conserved in evolution that have a central role in regulation of development in several tissues. We recently showed that one of them, *Ebf2*, is expressed in Schwann cells and that *Ebf2-/-* mice show a delay in myelination and a peripheral motor neuropathy.
Ebf transcription factors present an high level of sequence similarity and are able to homo and heterodimerize, suggesting a high level of functional redundancy. We therefore investigated the possible expression of the other *Ebf* members in peripheral nerves. We found that also *Ebf1* and *Ebf3* are expressed in Schwann cells and, interestingly, that *Ebf1* is upregulated in *Ebf2-/-* sciatic nerves, while *Ebf3* expression seems to be unaffected.
To better understand the role of the three *Ebf* genes in peripheral nerve myelination and to avoid redundancy, we generated a lentiviral vector expressing a triple shRNA to simoultaneously downregulate the three genes in an in vitro model of myelination, such as dorsal root ganglia (DRG) organotypic cultures.
Infection was performed 2 days after dissection at different concentration of virus (Multiplicity of Infection, MOI) and myelination was induced with ascorbic acid, allowing embryonic Schwann cells to differentiate and form regular myelin sheath around sensory axons. The expression of GFP reporter observed by fluorescence microscopy showed that an shRNA lentiviral delivery of 5 viral particles per cell (MOI 5) presents an high percentage of infected cells (about 100%) without relevant toxicity.
Gene expression analysis by Real Time qPCR showed a strong downregulation for the three *Ebf* genes compared to controls at different time point during the 30 days in vitro (div) of the experiment. As a consequence of *Ebf* family silencing we found a similar downregulation of Myelin protein zero (P0) transcript, which encodes one of the main protein of the myelin sheat. Western blot analysis on DRG lisates at 20div and 30div confirmed the reduction of P0 also at the protein level compared to controls.
Immunofluorescence staining for Myelin Basic Protein (MBP), another important myelin protein, in order to evaluate the integrity of the myelin sheat showed a strong reduction in the number of myelinated fibers and the presence of segmental dismyelination in DRG cultures infected with *Ebf* shRNAs compared to controls.
These findings suggest that *Ebf* genes cooperate in regulating myelin sheat formation in the early stages of development. Further studies will help to understand if the observed myelin defects are due to a specific Schwann cell impairment, a neuronal signalling defect or both, and will be achieved by setting up cocultures where *Ebf* silencing will be performed separately in Schwann cells or in neurons.

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Characterization of the arm morphology during embryonic development of Octopus vulgaris

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The Cephalopod arm crown is an evolutionary novelty within the phylum of molluscs, which derived from the muscular foot of a monoplacophoran-like ancestor and shows many specializations and adaptations within their own class. The Octopus arm crown consists of eight prehensile and amenable arms, which are composed of a three-dimensional combination of longitudinal and transverse muscle fibers differently combined with the mesh of connective tissues. This so-called muscular hydrostat provides the arm with skeletal support and allows the animal to move its arm in all directions, stiffen it and use the entire length of arm for complex object manipulations. The motor activities of the arms are controlled peripherally by the elaborated arm nervous system composed by two axial nerve cords and a chain of ganglia running for the entire length of each arm. Very little is known about the embryonic development of the Octopus arm crown and the generation of the various muscle types and connections between the arm tissues. The aim of our study is to analyze the development of the Octopus arm crown in order to understand the mechanism with which this complex structure is built. Here we present a description of Octopus arm development using standard histological techniques, focusing on arm bud formation, elongation and differentiation. In order to get a deeper insight into the formation of specific cell types we provide preliminary data on muscle and nervous system formation using an immunohistochemical approach. The information provided by our study will set a basis for further embryological studies on arm development and might give insights into the evolution of the cephalopod arm crown from the muscular foot of more basal molluscs. Furthermore, by comparing the development of the Octopus arm crown to the pathway of regeneration of the adult arm structure we might identify shared mechanisms of axes formation and cell patterning in the arm generation.

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The transcription factors Sp1 and REST interplay to modulate Synapsin I gene expression

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Synapsin I is a neuro-specific phosphoprotein associated with the cytoplasmic surface of synaptic vesicles, which plays a crucial role during axonogenesis and synaptogenesis as well as in synaptic transmission and plasticity. Abnormalities of *SYN1* gene expression have been associated with the onset of several neuropsychiatric diseases, including epilepsy, autism or schizophrenia. Thus, to reveal the mechanisms modulating *SYN1* gene expression is a central point in the understanding of the molecular events triggering these pathologies.
To further characterize the mechanisms modulating the *trans*-activation of the *SYN1*promoter, we subjected this gene region to a computational analysis which predicted, amongst other factors, a high number of *cis*-sites for the ubiquitous transcriptional factor Sp1. We demonstrated the physical interaction of Sp1 with the *SYN1* promoter and revealed, for the first time, that Sp1 plays an important role in promoting *SYN1* gene transcription. Moreover, we demonstrated that Sp1 activity on *SYN1* promoter is modulated by the RE 1 silencing transcription factor (REST), showing a strict functional interplay between these nuclear proteins. Using gene reporter assays and chromatin immunoprecipitation approaches we demonstrated that REST directly inhibits the Sp1 positive action, resulting in down-regulation of the *SYN1* gene.
Interestingly, the *SYN1* 5' flanking region was predicted as a CpG island, and a specific methylation of Sp1 *cis*-sites was found in cells and tissues not expressing synapsin I. Thus, we propose a model whereby methylation of Sp1 *cis*-sites inhibits Sp1-mediated transcription.
In conclusion, our results introduce Sp1 as a fundamental activator of basal *SYN1* gene expression, whose activity is modulated by the neural master regulator REST. Future experiments are ongoing to further investigate the role of epigenetic modifications on Sp1-mediated *SYN1 trans*-activation.

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Correlation between morphotype and phenotype of wild and chemoresistant colon cancer cells, by means of quantitative shape analysis

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Cell morphology roughly scales with malignancy and morphological, qualitative observations allow pathologists to correlate the shape the cells acquire with the neoplastic transformation they are experienced.
A quantitative parameter for characterizing complex irregular structures is the Normalized Bending Energy (NBE). NBE provides a global feature for shape characterization correspondent to the amount of energy needed to transform the specific shape under analysis into its lowest energy state.
We hypothesized that a chemotherapy resistant cancer cell line would experience a significant change in its shape, and that such a modification might be quantified by means of NBE analysis. We checked out the usefulness of a mathematical algorithm to distinguish wild and 5-fluorouracil (5-FU)-resistant colon cancer HCT-8 cells (HCT-8FUres). NBE values, as well as cellular and molecular parameters, were recorded in both cell populations.
Results demonstrated that acquisition of drug resistance is accompanied by statistically significant morphological changes in cell membrane, as well as in biological parameters. Namely, NBE increased progressively meanwhile cells become more resistant to increasing 5-FU concentrations. These data indicate how tight the relationships between morphology and phenotype is, and they support the idea to follow a cell transition toward a drug-resistant phenotype by means of morphological monitoring.

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Synapsin III regulates radial migration and morphological maturation of pyramidal neurons *in vivo*

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Synapsins (Syns) are phosphoproteins abundantly expressed in the brain. They localize at the presynaptic site where they associate with synaptic vesicles, thus regulating synaptic transmission and neuronal differentiation. While Syn I and II have partially overlapping functions, Syn III seems to have a distinctive role. Unlike Syn I and Syn II, which are expressed mainly in the adult brain, Syn III is highly expressed at early stages of neuronal development, and its level of expression decreases in mature neurons both in vitro and in vivo. The in vitro studies suggest that cultured hippocampal neurons depleted of Syn III have a significant reduction in neurite extension and enlargement of growth cones. However, Syn III KO mice present only mild phenotypes possibly due to compensation phenomena triggered by the chronic depletion of the protein. In order to study the effects of an acute alteration of the expression levels of Syn III in vivo, we took advantage of the in utero electroporation technique. We downregulated Syn III expression by means of RNA interference (RNAi) and found that this treatment caused defects in radial migration, morphological maturation and orientation of pyramidal neurons at early developmental stages. Then, we verified the specificity of the shRNA by expressing it together with an shRNA-insensitive Syn III cDNA (rSyn III). Under this condition, we completely rescued the phenotypes observed at the various developmental stages. Interestingly, overexpression of Syn III also led to defects in radial migration and pyramidal neuron morphology (but not orientation). Moreover, a mutant rSyn III non-phosphorylatable by cyclin dependent kinase 5 (Cdk5) was not able to rescue the defect in radial migration caused by Syn III downregulation. Taken together, these data suggest that Syn III activity, tightly regulated by expression restrictions and phosphorylation, is necessary for proper pyramidal neuron migration and maturation. This work for the first time shed light on the role of Syn III during brain development in vivo.

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Role of receptor lateral mobility in the neuronal computation

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Receptor diffusion has recently emerged as a key determinant in regulating the activity and the excitability of the neuron by rapidly controlling receptor availability in the post-synaptic density in the milliseconds range (Heine et al., 2008). Keeping in line with this idea, it is reasonable to consider that receptor diffusion can also modulate the cross-talk among synapses through the different conformational state of receptors (closed, open, desensitized), representing a new regulatory mechanism for fine integration of the signals.
In order to provide an experimental description of the role of diffusion in molecular computation in relation to the conformational state of the receptor we will perform Single-Particle Tracking (SPT) in combination with the optogenetic Photoswitchable Tethered Ligand (PTL) approach. SPT technique allows the tracking in real time of the motion and diffusion of ionic channels and receptors (Triller and Choquet, 2008). PTLs are used to remotely control protein function with light: in particular, our optogenetic tool is a genetically and chemically engineered ionotropic glutamate receptor (LiGluK2).
Moreover, we will explore the role of the activation of one (or few) neurotransmitter receptors in the neuronal electric activity. In fact, there are evidences that important biological events are initiated by the activation of a single molecule (for example phototransduction in vertebrate rods) and the nervous system represents, stores, processes and exchanges information also at a single molecule level.
The combined use of SPT and PTL will allow in-depth investigation of the role of single neurotrasmitter receptor molecules to the neuronal excitability.

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Epithelial mesenchymal transition traits in honey-driven keratinocyte wound healing

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Skin lesions generally heal rapidly and efficiently, but some pathological or severe wounds do not heal completely. Hence, great interest has been attracted by therapies providing healing acceleration and reducing wound-related complications. Honey has a number of properties that are believed to facilitate the healing process. Its acidic pH, H2O2 production, high sugar content, and specific plant-derived chemicals serve to inhibit microbial growth. However, little is known about mechanisms involved in honey-promoted wound healing. Given the overlapping phases of the wound healing process, this kind of remedy should affect at least two different processes before it can be said to have some scientific support for wound healing use. For this reason, we included in our experiments keratinocytes and fibroblasts, which play main roles in wounded skin repair.
By using in vitro scratch wound models we have shown an increase of wound closure induced by selected monofloral honey types (acacia, manuka, buckwheat) on both keratinocytes and fibroblasts. In addition, cell migration assays have revealed honey chemoattractant effects on both cell types.
Syndecan-4 and metalloproteinases (MMPs) are known to play essential roles in wound repair-linked cell migration. Such a body of evidence was fully confirmed in the present study, showing that all honeys share the ability of inducing the expression of syndecan-4, and in addition that MMP-9 is invariably the most stimulated metalloproteinase.
Cutaneous wound healing restores the epidermal barrier against external environment during a process called re-epithelialization. A key feature of re-epithelialization is the migration of cells under the stimulus of injury signals, representing an example of a cell differentiation process known as epithelial-mesenchymal transition (EMT). We first investigated whether honey exposure is able to induce pathways of cytoskeletal rearrangement in keratinocytes by means of a phospho-antibody array. The shared ability among honeys of activating CDK2 and FAK has suggested the occurrence of a shift towards a proliferative/motile phenotype.
Further evidence of EMT came from use of the Human EMT RT² Profiler™ PCR Array. Manuka honey induced few variations in terms of both up- and downregulation, whereas the regulatory patterns of the other two honeys were more complex. A common feature was a rise in MMP-3 expression, while acacia and manuka also induced MMP-9, confirming the above MMP antibody array data. Acacia and buckwheat honeys showed common up-regulation of known EMT markers like vimentin, HPRT-1 (also induced by manuka), and STEAP-1, along with the downregulation of keratin 14 and 19, two important markers of epithelial phenotype. Cadherin-2 was also upregulated and positively associated with vimentin expression. TGF-β was commonly downregulated by honeys, while components of the WNT family were activated by acacia and buckwheat, suggesting a role of these latter in honey-induced keratinocyte de-differentiation.
In conclusion, the results of our study suggest that the combination of honeys of different origin could allow to maximally exploit therapeutic properties such as antisepsis and tissue regeneration, thus yielding a possible solution to severe clinical conditions associated to diabetic wounds, chronic ulcers, and burns.

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Synaptic alterations induced by amyloid-β protein in experimental models of Alzheimer's disease

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Amyloid-β (Aβ) protein, generated by β- and γ-secretase-mediated cleavage of the amyloid precursor protein, has been found as the key molecule in the AD pathogenesis.
Low Aβ concentrations play critical physiological roles in synaptic plasticity (Puzzo et al., 2008, 2011) and activity-dependent regulation of synaptic vesicle release (Abramov et al., 2009), but abnormal accumulations lead to the self-assembly of neurotoxic Aβ oligomers, which interfere with synaptic function and cause neurodegeneration (refs. in Querfurth and LaFerla, 2010).
This study was conducted to investigate the pathogenic role of Aβ oligomerizzation, the effects of post-production Aβ modifications on synaptic alterations and the molecular mechanisms of Aβ-induced synaptic dysfunctions.
We found that a new synthetic molecule, named CLR01, protects neurons from Aβ42-induced neuro- and synaptotoxicity through a process-specific modulation of amyloid proteins' self-assembly both in vitro and in a 15-month old triple-transgenic mice. In the presence of CLR01 the Aβ-induced impairment of spontaneous and evoked synaptic transmission and long-term potentiation (LTP) was markedly rescued. Our data indicate that disruption of Aβ oligomerization is a promising therapeutic approach to prevent and/or minimize the synaptic dysfunction correlated to the cognitive decline observed in AD patients.
Although, Aβ assembly is critical to the protein's synaptotoxicity, we also investigated others factors contributing to Aβ42-induced synaptosis.
Aβ42 contains a single methionine residue, in position 35 (Met35), whose sulfur atom is highly subject to oxidation, a post-production event that markedly alters C-terminal hydropathy and thus affects Aβ assembly (Bitan et al., 2003). Indeed, Aβ42 in which the sulfur atom of Met35 has undergone a single oxidation to sulfoxide or a double oxidation to sulfone did not form Aβ42 trimers and tetramers which were found in wild-type Aβ42 (with reduced sulfur atom) as demonstrated Western-blot experiments with 6E10 antibody in.
Despite identical oligomer-size distribution of sulfoxide and sulfone Aβ42 analogues, these two Aβ preparations exhibited significant differences in synaptotoxicity, with Aβ42-sulfone synaptotoxic as wild-type whereas Aβ42-sulfoxide exhibited a significantly lower synaptotoxicity than the other analogues.
The differential synaptotoxicity of these peptides reflects their relative capacities to interact with the plasma membranes of neurons, to cross them and accumulate intracellularly, indicating that Aβ oligomerization is necessary but not sufficient for Aβ42-induced synaptic alterations.
The role of Aβ internalization was further investigated by electrophysiological recordings in autaptic microcultures of hippocampal neurons in which Aβ42 was loaded with the patch-pipette.
Finally, we found that a caspase-3-depending signaling pathway mediated the synaptic defects induced by Aβ42 after both extra- and intracellular peptide application.
Collectively, our findings contribute to a greater understanding of mechanisms underlying the Aβ-induced synaptic failure that correlates with cognitive impairment observed in AD.

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Transduction pathways involved in FGF2-induced calcium signals in cultured parasympathetic neurons

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Basic Fibroblast Growth Factor (bFGF or FGF-2) has been shown to promote neuronal survival and neurite outgrowth in dissociated neurons from embryonic (E7-E8) chick ciliary ganglion (CG). The three main signal transduction pathways downstream the activated FGFR receptor are those mediated by MAPK, PI3-K and PLCγ. All of them are differentially involved in these bFGF-induced growth effects, but, while it has been shown that bFGF can elicit long lasting elevations in intracellular calcium concentration, $[Ca^{2+}]_i$, the role of the three pathways in this process has not been elucidated. Here we show, by means of pharmacological inhibitors, that all three are involved, at a different extent, in the generation of the $[Ca^{2+}]_i$ increase induced by bFGF. In particular, inhibition of the PLCγ pathway, in addition to reducing the number of responsive cells, induces, in a significant population of cells, basal calcium oscillations even in the absence of the growth factor and downregulates voltage-dependent calcium channels. This complex behaviour can be due to a perturbation in PIP_2 levels at the plasmamembrane.

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Higher density of NMDAR1 subunit in prefrontal cortex of an animal model of hyperactivity and attention deficit: expression, functional relevance and plasticity

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The neural substrates of complex behaviours and neuropsychiatric problems, such as schizophrenia, endogenous depression and Attention-Deficit Hyperactivity Disorder, can be studied in genetic model systems. The Naples High-Excitability (NHE) rats show hyperactivity and impaired non-selective and selective attention, that are associated with increased activity in the mesocortical dopamine (DA) branch (1). DA, Serotonin, Norepinephrine and Histamine modulate Glutamate neurotransmission at Prefronto-Striatal interface, leading to correct regulation of executive functions. A Subtractive library study on Prefrontal Cortex (PFC) showed that 51 out of 200 clones were hyper expressed in NHE compared to Random Bred control rats (NRB). Among these there was the NMDAR1 glutamate receptor gene. The aim of these studies was three fold: i) to investigate the level of RNA expression for the NMDAR1 subunit of the glutamate receptor in the PFC, by western blot analysis; ii) to clarify functional relevance of the higher NMDAR1 density, by behavioural analysis after acute and subchronic treatment with the non-competitive antagonist MK801; iii) to study the plasticity of the NMDAR1 system by behaviour analysis and NMDAR1 expression after subchronic MK801 treatment in NHE and NRB rats. Therefore young-adult male rats of NHE line and NRB were used. In exp 1 western blot analysis was carried out on left and right hemisphere separately. In exp 2 acute MK801 (0.0, 0.0001, 0.001, and 0.01 mg/kg) was given intraperitoneally (i.p.), finally in exp 3, subchronic MK801 (0.01 mg/kg) was given i.p. daily over 14 days. Thus NHE and NRB rats were than exposed to a spatial novelty (Làt-maze) and horizontal (HA) and vertical (VA) activity were monitored. Results. Exp1: western-blot analysis confirmed the higher level of NMDAR1 subunit expression in the left PFC of NHE rats, as previously demonstrated with subtractive library. In particular, the higher density of NMDAR1 in NHE accounted for 30% of NRB rats. Exp 2: acute MK801 increased HA in both NHE and NRB rats at the dose of 0.01mg/kg only. In contrast, MK801 exerted an inverted-U action upon VA frequency and the highest dose decreased its duration in NRB rats only. Exp 3: subchronic MK801 increased the density of the NMDAR1 subunit in the PFC of NRB rats by 78%. Moreover, the latter was not associated with a higher activity level. In conclusion, the higher density of the NMDAR1 subunit in the PFC of NHE rats is not directly related to hyperactivity, but could be involved in the altered non selective attention. This in turn, may reveal an unbalanced glutamate control of prefrontal output leading eventually to distorted prefronto-striatal neurotransmission.

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1 L.A. Ruocco, A.G. Sadile U.A. Gironi Carnevale. Modeling the mesocortical variant of adhd: the Naples High-Excitability rats. Editors: 2009 Nova Science Publishers, Inc.

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Rat pial microvascular remodeling after transient middle cerebral artery occlusion: role of nitric oxide

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The biological processes underlying stroke are complex; recently research shifted from a purely neurocentric focus to a more integrated view, wherein dynamic interactions between all cell types contribute to function and dysfunction in the brain. The so-called "neurovascular unit" provides a conceptual framework that emphasizes cell-cell interactions among neuronal, glial, and vascular elements. Experimental data have shown that these cell-cell signaling mechanisms may also mediate parallel processes of vascular remodeling during stroke recovery involving cellular changes, production or degradation of extracellular matrix, dependent on dynamic interaction among growth factors, vasoactive substances and hemodynamic stimuli. Few study have been focused on the spatial and temporal dynamics of post-stroke cerebral microvascular rearrangement and on the endothelial factors implicated. Emerging evidences suggest that the main factor involved in vascular angiogenesis appear to be Vascular Endothelium Growth Factors (VEGF) promoting NO production and inducing eNOS and iNOS expression in in vitro vascular endothelial cells.The aim of this study was to assess the in vivo structural and functional remodeling of pial arteriolar networks in rats submitted to transient middle cerebral artery occlusion (MCAO) at different time-intervals of reperfusion. In particular eNOS, nNOS and iNOS expression was evaluated. MCAO was induced, for two hours, by the intraluminal filament method. Pial microcirculation was observed by fluorescence microscopy thecnique throught two closed cranial windows implanted above the left (affected emisphere) and rigth parietal cortex. Geometric characteristics of pial arteriolar networks, permeability increase, leukocyte adhesion, eNOS, nNOS and iNOS expression were analyzed after MCAO and 1 hour or 1 or 7 or 14 or 28 days of reperfusion. MCAO and 1 hour of reperfusion caused marked microvascular changes in pial networks of the affected hemisphere. The necrotic core was devoid of vessels, while penumbra area presented few arterioles, capillaries and larger venules. Microvascular permeability and leukocyte adhesion were pronounced. After 7 days of reperfusion, in affected hemisphere, pial arterioles were organized in arteriolar anastomotic arcades, overlapping the ischemic core and in penetrating pial arterioles. This vascular remodeling was complex until 28 days of reperfusion compared with microvasculature observed in contralateral hemisphere or in sham-operated rats. At 1, 7, 14 and 28 days of reperfusion eNOS protein concentration significantly increased in the cortex and striatum of the affected hemisphere compared with contralateral hemisphere or sham-operated ipsilateral hemisphere and peaked after 7days of reperfusion. nNOS expression increased in the ipsilateral cortex at 1 day of reperfusion while a further increase was observed at 7 days of reperfusion in ipsilateral striatum compared with contralateral one or sham-operated ipsilateral hemisphere. iNOS significantly increased in ipsilateral cortex and striatum compared with contralateral hemisphere or sham-operated ipsilateral hemisphere only at 1 and 7 days of reperfusion. In conclusion, after transient MCAO pial networks were rearranged through anastomotic arterioles. These arcades were likely new vessels originating from penumbra preexisting arterioles. Remodeling mechanisms appear to be accompanied by higher expression of eNOS likely modulating angiogenesis in vivo.

Therefore, it is reasonable to suggest that VEGF involvement in microvascular rearrangement is affective through the activation of eNOS.

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Local, use-dependent changes in the waking EEG after prolonged wakefulness

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Background and Objectives
In humans, prolonged wakefulness is commonly associated with increased sleepiness and impairment in behavioral performance. Previous work has shown that sustained wakefulness is associated with a progressive, homeostatic increase in EEG power density in the theta/alpha frequency range. In this study we investigated whether specific behavioral manipulations targeting distinct cortical areas could locally regulate these changes during prolonged wakefulness.

Methods
Sixteen subjects (right-handed, 22+/-2.7y, 7 females) participated in two prolonged wakefulness experiments (24-h). During each experiment, subjects were exposed to six 2-h bouts of either audiobook listening (AB) or driving simulator playing (DS). These tasks were chosen as previous imaging studies showed that speech listening tasks and driving simulation tasks involve different cortical areas, with the former activating the left fronto-temporal cortices and the latter activating occipito-parietal networks. Resting waking high density EEG (256 channels) with eyes open was recorded for 4 min before and after each task bout, preceded by subjective sleepiness evaluation and a 10-min psychomotor vigilance test (PVT).

Results
After 24-h prolonged wakefulness, both tasks induced a global homeostatic increase in the resting waking EEG power in the theta/alpha frequency (6-9 Hz), concurrent with increased subjective sleepiness and PVT reaction times ($p<0.05$, paired t-test). Analysis contrasting topographical changes between the two experiments further demonstrated a task-specific effect on the theta/alpha frequency, with a local power increase over the left fronto-temporal derivations for the AB experiment and over the parietal-occipital derivations for the DS experiment ($p<0.05$, SnPM cluster test).

Conclusion
Our results support the notion that prolonged engagement in a particular task leads to a local, use-dependent enhancement of sleep propensity in the task-related regions compared with other regions. The regional difference in the homeostatic sleep regulation may result from disproportionate activation of cortical neuronal circuits during prolonged wakefulness.

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Hypertension-linked mutation of α-adducin increases CFTR surface expression and activity

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CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) activity and localization are influenced by the cytoskeleton, in particular by actin and its polymerization state. In this study we demonstrate that the expression of the hypertensive mutations of adducin (G460W-S586C in humans, F316Y in rats), an actin binding cytoskeletal protein, leads to a functional modification of CFTR activity and surface expression. The experiments were performed on HEK cells cotransfected with CFTR and the human wild-type or G460W mutated adducin. In whole-cell patch-clamp experiments, both the CFTR chloride current and the slope of current activation after forskolin addition were significantly higher in HEK cells overexpressing the G460W adducin, a higher plasma membrane density of active CFTR was confirmed by cell-attached patch-clamp experiments. By Western blot, an increase of the plasma membrane CFTR expression, with a modification of the channel glycosylation state, was observed in the presence of the mutated adducin. CFTR-adducin interaction and CFTR trafficking have been investigated in order to clarify the mechanism by which adducin could influence CFTR activity and expression. FRET and immunoprecipitation experiments revealed a faint interaction between the two proteins, both FRAP and photoactivation experiments indicate that CFTR is more retained in the plasma membrane in the presence of G460W adducin.
The increase in CFTR channel activity is possibly related to the modulation by adducin and could be the consequence of an altered membrane turnover leading to a retention of the channel in the plasma membrane. Since CFTR is known to modulate the activity of many others transport systems, the increased surface expression of the channel could have consequences on the whole network of transport in the kidney tubule.

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The physiological and pathological role of miR-101 in human colon cells

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MicroRNAs (miRNAs) are small non-coding RNA molecules that control gene expression at post-transcriptional level by reducing the stability and/or translation of target mRNAs. A growing number of reports have implicated hsa-miR-101 (miR-101) as an important regulator of many human genes, including oncogenes, involved in the control of cell phenotype and behaviour. Interestingly, miR-101 expression is tissue-specific and its dysregulation has been associated to several human pathologies, particularly cancers. In 2009, we first demonstrated that miR-101 is a direct repressor of cyclooxygenase-2 (COX-2) mRNA translation in human cells and we also showed an inverse correlation between miR-101 and COX-2 expression in colorectal cancer (CRC) specimens and cell lines. Given these observations, our aims are to determine the role of miR-101 in human cell pathophysiology and to better characterize miR-101 gene regulation. In this regard, we found that miR-101 expression correlates with tissue differentiation in human colon mucosa and that different biological events underlying CRC malignancy promote miR-101 repression, whereas the restoration of physiological miR-101 levels impairs the malignant behaviour of CRC cells. Moreover, we collected preliminary data on miR-101 putative promoter(s), in order to elucidate the molecular events that regulate the transcription and expression of this important molecule. This study represents an important advancement in the understanding of the molecular and cellular mechanisms that control the phenotype of human colon cells. Also, miR-101 could be an important molecular target for CRC prevention and therapy and could be considered a candidate cancer biomarker for CRC diagnosis and prognosis.

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Adult neurogenesis and age-modulated microRNA expression in the brain of the short-lived teleost *Nothobranchius furzeri*, an emerging model for ageing studies

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We studied adult neurogenesis in the short-lived annual fish *Nothobranchius furzeri* and quantified the effects of aging on the mitotic activity of the neuronal progenitors and the expression of glial fibrillary acid protein (GFAP) in the radial glia.
The distribution of neurogenic niches is substantially distributed along the entire rosto-caudal extent of the ventricle and adult stem cells generate neurons which persist in the adult brain.
We demonstrated in *N. furzeri* an age-dependent decay in adult neurogenesis. Using unbiased stereological estimates of cell numbers, we detected an almost 5-fold decrease in the number of mitotically-active cells in the optic tectum between young and old age. This reduced mitotic activity is paralleled by a reduction of doublecortin labeling (DCX, known to be transiently expressed in newly generated neurons)
Finally, we detected a dramatic up-regulation of GFAP in the radial glia of the aged brain. This upregulation is not paralleled by a similar up-regulation of S100B and Musashi-1, two other markers of the radial glia and can be recognized as a gliosis event.
In summary, the brain of *N. furzeri* replicates two typical hallmarks of mammalian aging: gliosis and reduced adult neurogenesis.
Some microRNAs showed a differential expression in the *N. furzeri* brain during ageing, as demonstrated by sequencing and qPCR data (Baumgart et al., 2012). We performed ISH experiments on young and old brains for three microRNAs from the set of those with age-regulated expression – miR-15a, miR-20a (a member of the miR-17~92 cluster) and miR-101a - combined with PCNA staining to visualize proliferative regions. According to the previous sequencing and qPCR results, we have found a clear age-dependent modulation in all three microRNAs:
- mir15a increases with age: its expression is stronger in old brains, with a higher concentration in correspondence of the proliferative niches of telencephalon and optic tectum
- mir20a is concentrated in the neurogenic niches and decreases with age, showing a stronger expression in young brains.
- mir101a increases with age, showing a stronger expression in the telencephalic region of old brains and is excluded in the neurogenetic niches.
In addition, we performed double-labelling of miR-124 and miR-9 with S100 as marker of glial cells and confirmed that expression of miR-124 is specific for neurons and expression of miR-9 is enhanced in radial glia cells.
We conclude that *N. furzeri* is an appropriate model for investigations of neuronal stem cell aging and that miR-15a and miR-20a show opposite regulations in the neuronal stem cells and may have opposite actions on neuronal stem cell aging.

POSTER

1) Sheila Maria Alvarez Fernandez (University of Milano): Surface ADAM10 protein elicits a serological immune response in colorectal cancer patients.
2) Marina Angelini (University of Milano): Positive correlation between CLIC1 expression in the plasma membrane and human glioma aggressiveness.
3) Alessandro Arena (Vita-Salute San Raffaele University, Milano): Analysis of the effects of anesthetics on the activity of rat visual cortex.
4) Marco Barbariga (University of Milano): Cerebrospinal fluid ceruloplasmin oxidation induces NGR motifs deamidation with gain of pro-adhesive function.
5) Thorsten Becker (University of Verona): Endocannabinoids control the GABA-ergic neurotransmission on orexinergic neurons in the lateral hypothalamic area in obese mice.
6) Oscar Gerardo Brenes Garcia (University of Torino): *In vitro* monosynaptic circuits of *Helix* neurons as an experimental model to study synapsin knock-down.
7) Carlo Bruttini (University of Milano): Hand immobilization affects arm and shoulder postural control.
8) Pasqua Cancellara (University of Padova): Structure and function of biceps and quadriceps muscles in elderly subjects.
9) Nicola Maria Carucci (Scuola Normale Superiore, Pisa): The contribution of early inflammation to neurodegeneration in anti-nerve growth factor mice.
10) Elisa Castaldi (University of Firenze): Selectivity to spatial phase of chromatic cortical mechanisms: an fMRI study.
11) Giulia D'Urso (The Italian Institute of Technology, Genova): Role of layer V principal neurons in the regulation of the integration property of cortical columns in the mouse somatosensory cortex.
12) Cristina Deflorio (University of Roma La Sapienza): Effect of riluzole on human muscle voltage-gated sodium currents in ALS myotubes.
13) Gabriele Deidda (The Italian Institute of Technology, Genova): Early-depolarizing GABA controls critical period plasticity in the rat visual cortex.
14) Federico Del Gallo (University of Milano): A mouse model of fatal familial insomnia (FFI): REM sleep reduction in transgenic mice.
15) Annarita Di Mise (University of Bari): Constitutively active variants of the calcium-sensing receptor: parallel adaptive feedback to explain the molecular basis of the gain of function.
16) Michael Di Palma (University of Urbino): Motor activity affects adult skeletal muscle re-innervation acting via Trk receptors.
17) Matteo Fecchio (University of Milano): Slow waves evoked by transcranial magnetic stimulation reflect a cortical downstate.
18) Claudia Fuchs (University of Bologna): APP-dependent neurogenesis impairment of neural precursors from the Ts65Dn mouse, a model for Down sindrome.
19) Adriana Carol Eleonora Graziano (University of Catania): Molecular mechanisms involved in psychosine-induced apoptosis.
20) Placido Illiano (The Italian Institute of Technology, Genova): Generation of humanized Dopamine Transporter Knock-in mouse strains carrying loss-of-function mutation as a model of human Dopamine Transporter Deficiency Sindrome.
21) Shahrzad Latifi (The Italian Institute of Technology, Genova): Presynaptic targeting of optogenetic probes.
22) Rosaliana Libro (University of Pisa): Role of hypoxia in the regulation of microvesicles-derived microRNAs in colorectal cancer cells.
23) Eleonora Margheritis (University of Insubria): Characterization of lysine - containing dipeptides transport by different PepT1 isoforms expressed in *Xenopus laevis* oocytes.

24) Serena Milano (University of Bari): Statins treatment increases AQP2 plasma membrane expression in vitro and in vivo: potential usefulness in the treatment of nephrogenic diabetes insipidus (NDI).
25) Melania Melis (University of Cagliari): Do salivary proteins influence bitter taste to 6-n-propylthiouracil (PROP) in humans?
26) Tatiana Moro (University of Padova): Gender differences in maximal strength improvement and muscle fiber characteristics after 8 weeks of resistance training.
27) Emanuele Murana (University of Roma La Sapienza): Transient increase in neuronal chloride concentration by soluble factors released from glioma cells.
28) Marta Orlando and Erica Tagliatti (The Italian Institute of Technology, Genova): Synapsin function in the regulation of the synaptic vesicle cycle in presynaptic terminals of hippocampal neurons.
29) Marco Pellegrini (University of Roma Tre): Does a different susceptibility to endocrine disruptors between sexes exist? The example of VSMC motility.
30) Andrea Pigorini (University of Milano): Neuronal downstates and cortical breakdown of causality during NREM sleep: an intracerebral study in humans.
31) Roberto Ripa (Scuola Normale Superiore, Pisa): Genetic methods to manipulate brain aging in the short-lived fish Nothobranchius furzeri: an novel model species for aging studies.
32) Valeria Rossetti (University of Milano): Oxidative stress of respiratory cells induced by H2O2 or cigarette smoke extract is reduced by the subministration of S-CMC-Lys.
33) Marco Segatto (University of Roma Tre): The regulation of emotional aspects of behaviour: a new role for cholesterol biosynthetic pathway in the central nervous system.
34) Luisa Speranza (University of Napoli Federico II): Serotonin receptor 7 (5-Htr7): a key-regulator of neurite outgrowth in telencephalic neurons.
35) Fiorenza Stagni (University of Bologna): Dendritic pathology and connectivity can be rescued by pharmacotherapy with fluoxetine in the Ts65Dn mouse model of Down sindrome.
36) Elisa Tavazzani (University of Pavia): Electrophysiological evidence for potassium accumulation between type I hair cells and calyx terminals in mammalian crista ampullaris.
37) Silvia Torretta (University of Bari): NKCC2 trafficking and activity: the role of interacting proteins.
38) Concetta Treno (Second University of Napoli): A neurogenetic approach to the study of non spatial attention in mice and rats.
39) Giovanna Trinchese (University of Napoli Federico II): Diet supplementation with donkey milk up-regulates liver mitochondrial uncoupling, reduces energy efficiency and improves anti-oxidant and anti-inflammatory defences in rats.
40) Francesca Tullio (University of Torino): Cardioprotection by postconditioning in experimental models of cardiac hypertrophy: spontaneously hypertensive and nandrolone-abuse rats.
41) Alessandra Vollero (University of Insubria): Relationship between temperature and kinetic properties in rabbit intestinal oligopeptide cotransporter PepT1.

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Surface ADAM10 protein elicits a serological immune response in colorectal cancer patients.

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In cancer the immune system is activated in response to aberrant qualitative and quantitative expression by tumor cells of certain proteins named tumor-associated antigens (TAAs). In a previous study we investigated TAAs inducing auto-antibody by exploiting the patients serological reactivity on the colorectal cancer (CrC) surface membrane proteoma and we identify a reactivity against ADAM10.
ADAM10 is a disintegrin metalloprotease with a potential role in tumor progression and invasion, being able to release or activate specific membrane due to its sheddase activity, and to digest the extracellular matrix.
A serological screening on purified ADAM10 was performed by Western blot in a testing cohort of CrC patients (n=57) that showed a significant presence of immunoreactivity compared to control sera (Ctrl; n=39). This indicated that an immune-response directed against ADAM10, was elicited in CrC patients, in particular in the advanced stages of the disease when regional lymphnodes are infiltrated by the tumor. Immunohistochemistry showed ADAM10 expression in the transformed epithelia of some patients and in particular in budding area of the tumor invading the stroma. Validation cohorts of CrC patients (n=50) and Ctrl. (n=50) has been tested. Also sera from pancreas adenocarcinoma (PC; n=49) and breast cancer patients (BC; n=43). The results confirmed that the serological reactivity against ADAM10 is a feature of CrC patients shared also by PC patients and by BC. To define whether anti-ADAM10 reactivity may be a marker for other tumor types, sera from B-CLL (n=59) and Multiple Myeloma (MM; n=46) patients have been tested. Results indicated that the presence of anti-ADAM10 antibodies in the patient sera is not significant in B-CLL and MM.
The presence of auto-Ab against ADAM10 in the sera of patients might be propose as biomarker candidate for carcinomas of epithelia origin, discriminating, in the case of colon cancer, early and late stages

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Positive correlation between CLIC1 expression in the plasma membrane and human glioma aggressiveness.

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Glioblastomas (GBMs) are brain tumors composed by two cell types: cancer stem cells (CSCs), which are a small population able to self-renew and generate progeny, and bulk cells, consisting of a larger population committed to a precise fate. Glioblastomas are aggressive tumors because of CSC brain infiltration efficiency and their resistance to chemotherapies. Therefore, CSCs represent the most tumorigenic component of GBM and we have focused our efforts on this small population of cell. Several forms of glioblastomas exhibit a level of Chloride Intracellular Channel 1 (CLIC1) expression higher than in normal brains. CLIC1 is a protein mainly localizes in the cytoplasm and in the nucleoplasm and, in stress conditions, it is able to translocate into plasma and nuclear membranes where it acts as a Cl- permeability. We are specifically investigating four human glioblastomas (fourth grade gliomas), which express CLIC1 which functionally express CLIC1 to understand the role of this protein in the infiltration process following CLIC1 silencing. Human glioblastoma biopsies were cultured in a medium selecting for CSCs that grow as neurospheres. By knocking down CLIC1 expression using siRNA lentiviral infection (siCLIC1), we found that CLIC1-deficient cells migrated about 50% less efficiently than cells treated with siRNA for luciferase (siLUC, control) in Boyden chamber assays. To determine whether this phenotype results from the lack of CLIC1 plasma membrane expression, CLIC1-mediated currents were estimated by using a specific inhibitor (IAA94, 100 µM) in voltage clamp experiments from perforated patches. We constantly record a IAA94-sensitive current in siLUC cells that was absent in siCLIC1 cells. Moreover, in non-treated CSCs we estimated the relative abundance of CLIC1-mediated current compared to the other Cl- currents in the cell. By calculating the ratio IAA94-sensitive current over DIDS-sensitive current we found a positive correlation between the tumor aggressiveness and the relative abundance of CLIC1-mediated currents in the four human glioblastomas analyzed. Our results suggest that CLIC1 is involved in glioma cells. The on-going experiments aim to understand the mechanism that mediates tumor infiltration via CLIC1 functional expression as an ion channel. The investigation on the intracellular pathway involving CLIC1 will be useful to eventually identify other proteins or enzymes that, together with CLIC1, could represent possible pharmacological targets to hamper brain tumor expansion.

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Analysis of the effects of anesthetics on the activity of rat visual cortex

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Even if anesthetics are widely used in the medical practice to ensure the induction and maintenance of general anesthesia, there is still an unresolved issue which relates to their mechanism of action therefore to the nature of their molecular target site(s).Since a large number of very different chemical molecules are used in general anesthesia,this suggests that a large variety of molecular targets must exist. Some of these might affect axonal conduction and membrane excitability. In some cases, this action could either occurat the presynaptic level, modifying the neurotransmitter release and reuptake, or at the postsynaptic level, modifying the receptor sensitivity. These functional alterations might also affect one or more neuronal phenotypes for example by dampening the activity of the Glutamatergic circuits, or by enhancing the activity of GABAergic networks (or other inhibitory systems).Despite the underlying complexity of molecular targets, all general anesthetics induce a general and profound inhibition of cortical EEG activity while sparing evoked cortical responses. Based on these considerations, I decided to begin the investigation of the electrophysiological basis of the action of general anesthetics on the visual thalamus and on cortical circuits for vision in the rat. In this project I will compare the effects of different anesthetic molecules including sevofluorane, propofol and ketamine.Fot these experiments rats are curarized (one bolus of atracurium, 5 mg/kg, every 20 minutes) and mechanically ventilated by the insertion of a cannula in trachea (the oxygen and CO2 level are continuously monitored). In these experiments I record/will record: i) visual evoked potentials (VEPs) by superficial electrodes implanted in the skull; ii)intracellular visual cortex activity by sharp electrodes; iii) the pattern of activation of neurons in the LGN and cortex by the expression of the c-fos gene protein product known to report neuronal firing; iv) by applying the synaptic biosensor, GreenZip, which has been recently developed in my laboratory,I will record synaptic network activity with single synapse resolution. In these experiments, rats will be visually stimulated with light pulses (20 msec) or with alternating black and white horizontal stripes at constant overall luminance (2Hz reversal, 0.5 cycle/degree). In a set of preliminary experiments that I will present at the meeting, I have characterized the effect of sevoflurane, a volatile anesthetic . I found that sevoflurane increases in a dose-dependent manner both the amplitude and the latency of VEPs, while the general cortical electrical activity is reduced. Among the working hypotheses I'mexploring : i)an unbalance between inhibition and excitationin thalamic or cortical circuits by the anesthetic ; ii) an increase in the pool of available vesicles for visually evoked activity because of reduced background activity . At the meeting I will present an account on this work describing the methodological approach and my preliminary results.

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Cerebrospinal fluid ceruloplasmin oxidation induces NGR motifs deamidation with gain of pro-adhesive function

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Ceruloplasmin (Cp) enzyme protect tissues from oxidative damage oxidizing toxic Fe2+ to non-toxic Fe3+. In Parkinson's disease, if compared to other neurological pathologies, the Cp in the cerebrospinal fluid (CSF) shows a more acidic profile mainly due to oxidative modifications. This modifications results in a loss of ferroxidase activity possibly due to structural changes induced by oxidation, which in turn favor intracellular iron retention in neurons (Olivieri et al.,2011).
In the sequence of Cp are present Asn-Gly-Arg (NGR) motifs (N568 and N962) in which asparagine might spontaneously deamidate to aspartate or to L-isoaspartate during protein aging; furthermore, this deamidation might be accelerated under pathological oxidative conditions. Asn deamidation at NGR site may induce a gain-of-function, because the resulting isoDGR-motif (isoAsp-Gly-Arg) can mimic RGD-motif and recognize the RGD-binding site of integrins (Curnis et al., 2006).
In this project we are analyzing whether during protein aging under oxidative conditions the Cp's NGR motifs deamidation occurs and whether this results in a gain of pro-adhesive property.
We demonstrated that after simulated aging of Cp, only N568, which is external exposed on the molecule surface, can be deamidated, while the internal exposed one (N962) deamidate only when the protein is aged under oxidative environment, a condition that alters the secondary structure of the protein and that may expose the motif on the surface.
Our results indicate also that the conversion to isoDGR (which coincides with a loss of ferrosidasic activity) is able to confer to the Cp a binding ability to different integrins, in particular αVβ5 and αVβ6; interestingly, the aging under oxidative conditions increases and accelerates these adhesive features. Binding experiments have shown that the whole ox-Cp and its aggregates interact with integrin, but not its fragments.
Moreover, oxidized and aged Cp promotes the adhesion and spreading of epithelial, glial and endothelial cell lines, throughout the integrins engagement by the isoDGR motif as demonstrated both by the use of the protein L-isoaspartyl methyltransferase, an enzyme that physiologically converts isoaspartate to aspartate, and by molecular dynamics and docking modeling.
In conclusion, Cp aging under oxidative conditions results in NGR motifs deamidation with gain of binding ability to αVβ6 integrin.
Our efforts are now directed to study the biological consequences of the Cp adhesive properties and their possible role in pathological mechanisms.

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Endocannabinoids control the GABA-ergic neurotransmission on orexinergic neurons in the lateral hypothalamic area in obese mice

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Orexin-expressing (OX) neurons are solely found within the lateral hypothalamic area (LHA), from where they send projections throughout the brain, thereby modulating food intake. Food deprivation has been shown to enhance the excitatory input on OX neurons, this enhancement can be reversed by leptin administration. Furthermore, it was shown that the cannabinoid receptor type 1 (CB1) is present in the LHA and involved in the regulation of feeding. These findings imply that both the leptin and the endocannabinoid systems play a role in modulating the activity of OX neurons. In normal mice, the afferents onto OX neurons are mostly excitatory and modulated by endocannabinoids upon depolarization of the same neurons (the so-called “depolarization-induced suppression of excitation“, DSE).
We have recently observed that OX neurons synthesize the endocannabinoid 2-arachidonoyl glycerol (2-AG), and morphologically described a higher CB1-expressing inhibitory innervation of ob/ob neurons compared to wt.
In the same neurons we also performed whole-cell voltage-clamp recordings of miniature Inhibitory Postsynaptic Currents (mIPSCs, to estimate the amount of functional inhibitory inputs) and spontaneous Inhibitory Postsynaptic Currents (sIPSCs, to examine the effect of CB1 activation with the agonist WIN55,212-2 (WIN)). The mIPSC frequency in 5 and 9 week old ob/ob mice was 2 - 3 times higher than in their age-matched wt siblings. No difference was observed between pre-weaning wt and ob/ob mice. The administration of WIN was able to significantly decrease the sIPSC frequencies in 5 and 9 week old ob/ob mice. When the CB1 receptor antagonist AM251 was applied the effect of WIN could not be observed. sIPSCs amplitude was not affected, suggesting a presynaptic action of WIN. DSI of sIPSC frequencies was observed in ob/ob mice lasting about 10 s after a 5 s depolarization step to 0 mV. DSI was prevented by AM251. No DSI of sIPSCs was obtained from wt mice neurons.
These data provide a putative mechanism mediating the activation of OX neurons within the LHA.
In my PhD-project, I want to investigate the functional consequences of the increased inhibitory innervation in ob/ob mice on the activity of OX neurons compared to wt . To do so I will record the membrane potential and the firing rate. In order to leave the balance between excitatory and inhibitory inputs on OX neurons as unperturbed as possible, I will perform perforated patch-clamp recordings using gramicidin D. Based on our previous findings, I expect the membrane potential of ob/ob OX neurons to be more hyperpolarized and the firing rate to be lower than in wt. Moreover, activating the CB1 receptors with WIN, ob/ob OX neurons should depolarize and their firing rate should increase, whereas the opposite should be seen in wt OX neurons.

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In vitro monosynaptic circuits of *Helix* neurons as an experimental model to study synapsin knock-down

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Synapsins are a highly conserved family of synaptic vesicle-associated phosphoproteins, involved in the fine-tuning of synaptic transmission and remodeling. They have been functionally related to neurite outgrowth, synapse formation, and synaptic plasticity.
Experimental evidence has shown that synapsin mutations or deletions might be involved in the development of pathological phenotypes, including epilepsy. The study of mammalian knock-out models have clarified in some degree the role of synapsin in epileptogenesis, but the presence of different genes and isoforms, and the possible development of compensatory mechanisms in mammalian models have made difficult an accurate interpretation of these results.
In mammals, the synapsin family is composed by at less ten homologous proteins, generated from three distinct genes. Orthologues have been described in several organisms, such as mollusks from genus *Helix*. Invertebrate culture cell preparations are particularly advantageous for several reasons: identifiable neurons can be specifically isolated from their synaptic inputs, avoiding unspecific effects due to surrounding tissue, and both excitatory and inhibitory reliable monosynaptic connections can be formed *in vitro*.
The aim of this project is to develop an in vitro monosynaptic connection, as a reliable experimental model, in which synapsin gene has been knocked-down, in order to over-express human epilepsy-related mutated forms of synapsin and investigate their effects. Here, we present some preliminary results about neuronal dysfunctions correlated to the lack of synapsin.
Firstly, we developed two different antisense RNAs (asRNA) against the mRNA of *Helix* synapsin (helSyn), cloned into a plasmid able to constitutively overexpress these constructs upon intra-nuclear microinjection in cultured cells. Co-injected EGFP-plasmid was used as a reporter to evaluate the expression level.
In order to verify the efficacy of asRNAs , high-level expressing neurons were immunolabeled with a custom antibody against helSyn, in order to evaluate the presence of synapsin protein after 48 and 72 hours of antisense expression. In control group, we observed that synapsin was predominantly localized in varicosities along neurites, and its levels increased with time after plating. Conversely, in asRNAs over-expressing cells we found a statistically significant time-dependent decrease of immunostaining intensity levels, respect to control group, starting from 48h, thus confirming the effective loss of helSyn protein.On the basis of these results, we investigated the effect of synapsin knock-down on neurite outgrowth. asRNA overexpressing cells cultured in isolated configuration displayed a significant reduction of linear outgrowth that is directly correlated with the amount of expressed synapsin. In addition, preliminary analysis also suggests a time-dependent decrease in the number of varicosities.
The next steps include the analysis of the capacity of the asRNAs over-expressing cells to form an appropriate synaptic connection with its physiological target, the study of synaptic transmission and neurotransmitter release kinetics, and the morphological characterization of the varicose structures

by using electron microcopy. Finally, this model will be employed for the constitutive expression of human synapsin proteins carrying mutations related to epileptic phenotype, allowing the morphological and electrophysiological analysis of their effects.

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Hand immobilization affects arm and shoulder postural control

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Objectives: Neuromuscular effects of limb immobilization are widely reported in the literature, however, most papers describe changes in the motor pathways deserving the prime movers of the immobilized joint. Conversely, the present study investigates the effect of a short-term immobilization on the activation of both the prime mover and the associated postural muscles. It has been recently observed that when rapidly flexing the index finger, the forearm equilibrium is preserved thanks to postural adjustments that occur in arm and shoulder muscles prior to the movement onset (APAs). These postural adjustments are excitatory in Triceps Brachii (TB) and inhibitory in Biceps Brachii (BB) and Anterior Deltoid (AD). In this study we tested if and how a 12 hours immobilization affects the APAs development.
Methods: Subjects (n=10) were sitting on a chair with the right arm along the trunk, the elbow flexed at 90° and the prone hand in axis with the forearm. Starting with the index finger extended, subjects performed a rapid flexion (about 5-7 cm), repeated every 4s for 120 times. The metacarpo-phalangeal and elbow joints angles were recorded, as well as the EMG activity from the prime mover Flexor Digitorum Superficialis and from the above-cited postural muscles (BB, TB and AD). At the end of the session, the EMG electrodes were left in place and the fingers and wrist joints immobilized by a cast which was removed 12 hours later. We then repeated the whole protocol.
Results: Short-term immobilization significantly reduced the excitatory APA in TB and increased the inhibitory APAs in BB and AD. The movement amplitude and duration, as well as the magnitude of the prime mover activation were unchanged.
Conclusions: The overall motor impairment following immobilization of a joint may be partly due to APAs modifications in muscles acting on other non-immobilized joints of the same limb.

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Structure and function of biceps and quadriceps muscles in elderly subjects

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Aging and disuse are the two main conditions leading to skeletal muscle atrophy in human. The age-related fibre atrophy is heterogeneous, and also affects different fibre types differently. In this study we analysed skeletal muscle needle biopsies of elderly subjects (age 90-100), divided in 2 groups, one group still able to walk (W) and the other unable to walk (NW); two muscle biopsies (from biceps and quadriceps) were collected for each subject. The aim of this work was to compare muscles at single muscle fibre level, both in size and in mechanical performance. The following parameters were determined in single muscle fibres: cross sectional area (CSA), isometric force (Fo) and tension (Po), and Myosin Heavy Chain isoform composition.The results obtained showed that 1) CSA was greater in W group than in NW for both biceps and quadriceps muscles. Comparing different fibre types, the difference was more pronounced in 2A fibres. 2) there was no statistically significant difference in isometric tension (Po) between W and NW group in both muscle types. 3) Percentage distribution of fiber types pointed to a decrease in slow fibres in NW compared with W group in both muscles.Ours results indicate that fibre types respond differently to disuse age-related effects. It is, therefore, important to assess fibre type distribution in skeletal muscles, in addition to study whole muscles.

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The contribution of early inflammation to neurodegeneration in anti-nerve growth factor mice

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Inflammation has long been proposed as having a role in Alzheimer's disease (AD), although it remains unclear whether inflammation represents a cause or consequence of AD.
In previous studies we showed that deprivation of Nerve Growth Factor (NGF) determines a neurodegeneration in AD11 anti-NGF that goes well beyond the expected cholinergic deficit. Interestingly, we showed that at pre-symptomatic stages the genes that are more influenced by the expression of the anti-NGF transgenic antibodies are those related to inflammation. The objective of this study is to validate these findings, at protein and cellular level.
We focused on the evaluation of the expression of one marker of neuroinflammation, CCL5 and on morphological variations of astrocytes in AD11 mice and related controls. Region-specific biochemical analysis was performed not only against CCL5, but also against GFAP and β-amyloid expression. The morphology of astrocytes was related to variations of synaptic markers. In addition, since it has been reported that early inflammation and innate immune response play a role in neurodegeneration, we reasoned that breeding AD11 under pathogen free (MPF) conditions might influence the progression of neurodegeneration.
We found: (1) an early and marked atrophy of astrocytes, as detected using GFAP, which correlates with a decreased expression of synaptophysin and CCL5; (2) a slowing down of the onset and progression of the neurodegeneration in MPF AD11 mice.
Thus we concluded that astrocytes are precociously affected by NGF deprivation and postulate that these cells might play a significant role in the onset of the neurodegenerative phenotype in AD11 anti-NGF mice.

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Selectivity to spatial phase of chromatic cortical mechanisms: an fMRI study

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Physiological and psychophysical evidence suggests that segmentation of an equiluminant visual scene into distinct objects could be achieved by a local energy algorithm computation, which relies on combining even- and odd-symmetric receptive field mechanisms. We measured BOLD responses to Fourier amplitude-matched edge and line stimuli modulated either in luminance or in red-green (equiluminant) colour contrast.
Alternation between edge and lines stimuli, produced no activation of primary visual cortex. Only higher hierarchical areas, either along the dorsal pathway (caudal part of the intraparietal sulcus (CIP) and V3A and along the ventral pathway (V4) responded with a preference for edges to line stimuli. The activity elicited by stimuli modulated in luminance confirmed previous results [Perna et al, 2008, Journal of Vision, 8(10): 15, 1–15]. Overall the results suggest the existence of equal numbers of neurones with even and odd receptive fields, for both luminance and colour stimuli in V1, as well as a specialization both along the dorsal and the ventral pathways for the detection of edges, both colour and luminance.

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Role of layer V principal neurons in the regulation of the integration property of cortical columns in the mouse somatosensory cortex

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The mammalian somatosensory cortex is organized in functional modules, often referred to as columns. Sensory information reaches the column mainly through ascending thalamic fibers, is processed by local cortical microcircuits and then transferred to higher-order areas through cortico-cortico and corticofugal connections. Within the column, layer V cells represent one of the principal output neurons projecting to the ipsilateral and contralateral hemispheres as well as to various subcortical regions. This project will test the hypothesis that layer V-mediated output does not only transfer information to other brain areas but, by influencing the activity of neurons located in granular and supragranular layers, also provides modulatory feedback to the processing of sensory information within a cortical column.

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Effect of riluzole on human muscle voltage-gated sodium currents in ALS myotubes

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I am currently a PhD student (2nd year) of the Neurophysiology PhD Programme, at the department of Physiology and Pharmacology "Vittorio Erspamer" Sapienza University (Roma, Italy), under supervision of professor Fracensca Grassi. In addition to my PhD project concerning the study of muscle AChRs, I studied the action of riluzole on human muscle voltage-gated sodium currents.
Riluzole is the only drug currently registered to treat patients with Amyotrophic Lateral Sclerosis (ALS), a fatal disease caused by progressive degeneration of upper and/or lower motor neurons, resulting in muscle denervation. This drug affects the function of a variety of ion channels in neurones and possibly in muscle. Emerging evidence has unraveled that riluzole can inhibit neuronal and muscle voltage-gated sodium channels, but the precise mechanism of action of this drug remains largely unknown in skeletal muscle cells. Using patch-clamp techniques, we investigated the effect of riluzole on muscle voltage-gated sodium currents (VGSCs) in myotubes obtained in vitro from control denervated or ALS patients. Riluzole, at clinically relevant concentrations (1 μM), blocked VGSCs in both types of human myotubes, shifting the midpoint of the activation curve to more positive potentials, suggesting that myotubes need more depolarizing currents to trigger an action potential and reflecting a lower cell excitability. Moreover, in presence of riluzole the voltage dependence of fast inactivation was shifted to the left, increasing the number of channels that are in the inactivated state at a given membrane potential. Having characterized the effect of riluzole on total VGSCs, the next step was to test riluzole effect on TTX-resistant currents. Innervated adult skeletal muscle expresses only the Nav1.4 sodium channel isoform (TTX-sensitive) while, in denervated muscle a TTX-resistant sodium channel isoform (NaV1.5) is also expressed. TTX-sensitive isoforms are blocked by nanomolar TTX concentrations, while TTX-resistant isoforms show sensitivity to TTX only at micromolar concentrations. So TTX at the concentration of 100 nM was used, to isolate NaV1.5 currents by blocking NaV1.4 channels. Riluzole effect on TTX-resistant currents was similar to its effect on total sodium currents. These data indicate that riluzole, while apparently safe on synaptic transmission, may affect the function of sodium channels in ALS patients, with possible side effects like asthenia, but the biological consequences remain to be investigated. These data can contribute to understand the mechanism of action of riluzole, characterizing for the first time its action on muscle VGSCs, which will provide important data to understand the effect of this drug. These results will gain relevance if it is verified that muscle actively contributes to motor neuron degeneration in ALS, in agreement with the "dying back" model, according to which muscle denervation precedes neuronal death.

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Early-depolarizing GABA controls critical period plasticity in the rat visual cortex

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GABA, the main inhibitory neurotransmitter in the adult mammalian brain, plays a fundamental role for development and plasticity of the visual system. Conversely, GABA exerts depolarizing and mostly excitatory actions during early development due to high expression of the NKCC1 cotransporter. Interestingly, nothing is known about depolarizing GABA control of plasticity mechanisms in the visual system. Here, we interfered with depolarizing GABA action by treating rat pups with a NKCC1 inhibitor (bumetanide) or vehicle from P3 to P8 and examined rats at P35. We found that physiological parameters (visual acuity, contrast threshold and binocularity) developed normally. However, the critical period for ocular dominance plasticity was prolonged in bumetanide-treated animals, as shown by a persistent sensitivity to the effects of a brief monocular deprivation. Moreover, visual cortical slices from bumetanide-treated rats showed reliable LTP in response to theta-burst stimulation of the white matter. To explain the higher level of plasticity in bumetanide-treated rats, we analysed basal GABAergic transmission and the extracellular matrix, which normally limit experience-dependent plasticity. We found a reduction of both the inhibitory tone and the density of perineuronal nets-surrounded cells. These results demonstrate that early depolarizing GABA exerts a long-lasting modulation of the levels of plasticity in adulthood.

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A mouse model of fatal familial insomnia (FFI): REM sleep reduction in transgenic mice

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Introduction. Prion protein (PrP) is a glycoprotein anchored to plasma membrane and expressed in most cell types, including neuron and glia. The physiological role of PrP is still uncertain, however alterations in this protein lead to severe disorders, such as Creutzfeldt-Jakob disease (CJD) and fatal insomnia (FI). The D178N/M129 mutation of the PrP gene is linked to the familial form of fatal insomnia (FFI). FFI is a rare neurodegenerative disease characterized by major alterations in sleep-wake cycle, vegetative control and circadian rhythms (Montagna et al, Neurology, 2: 167-76, 2003). In 2009 an animal model of FFI has been developed but no polygraphic evaluation of sleep-wake behavior was performed.
Aim of this study was to polygraphically investigate the sleep-wake patterns of transgenic mice carrying the murine homolog of the PrP mutation associated to FFI.

Materials and Methods. 12-months old male mice of four strains were instrumented for chronic polygraphic recording of sleep-wake activity, according to standard techniques. Strains were: i) C57BL/6J, wild-type mice (WT), as control strain (n=8); ii) PrP knockout (KO) mice (n=10); iii) mice expressing both wild-type and mutant PrP ($FFI^{+/0}$, n=8); iv) mice expressing only mutant PrP ($FFI^{0/0}$, n=9). Animals were maintained under a 12-12h light-dark cycle, at a constant temperature of 26±1°C. After recovery from surgery, EEG and gross body activity were recorded for 24h in undisturbed conditions.

Results. i) In mice expressing only the mutant PrP ($FFI^{0/0}$), analyses of the percentages of time spent in different vigilance states revealed that, during the light period, the total amount of REM sleep was significantly reduced to about half of that observed in other strains ($p \leq .001$, mean±SE: $FFI^{0/0}$: 4.17±0.72%; WT: 8.16±0.55%; KO: 8.39±0.53%; $FFI^{+/0}$:7.76±0.72%). The reduction of REM sleep was due to a decrease of the number of REM bouts ($p \leq .001$). Additionally, $FFI^{0/0}$ showed an impaired circadian rhythm of gross body movement (light vs. dark: t= -2.162, $p > .05$).
ii)Both transgenic mouse strains ($FFI^{+/0}$ and $FFI^{0/0}$) showed a significant increase in the number of transitions between vigilance states compared to control mice (mean±SE, $FFI^{+/0}$ vs. WT: 13.05±3.79, $p = .010$; $FFI^{0/0}$ vs WT : 18.37±3.68, $p \leq .001$). iii) A pathological activity, during the overall recordings, was noted in EEG patterns of both transgenic mouse strains. $FFI^{+/0}$ and $FFI^{0/0}$ showed, in a different percentage, bursts of high voltage polyphasic complexes with frequency peaking at about 7 Hz. Such activity was not detected in the other two strains (KO and WT).

Conclusions. These findings suggest that REM sleep and circadian rhythms are impaired in transgenic mice carrying the murine homolog of the PrP mutation linked to FFI. The transgenic mice used in this study represent an useful animal model of FFI and give new insights on the physiological role of PrP. The co-expression of wild-type and mutant PrP influences the phenotype, since sleep amount was not reduced in $FFI^{+/0}$. This observation also suggests that the expression of wild-type PrP may protect against changes produced by mutated PrP.

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Constitutively active variants of the calcium-sensing receptor: parallel adaptive feedback to explain the molecular basis of the gain of function

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Here, we analyzed the contribution of constitutively active variants of the Calcium Sensing Receptor (CaSR) to our understanding of some aspects of CaSR physiopathology. We combined biochemical and functional studies in cells expressing CaSR variants conferring a gain of function or loss of function to the receptor.
HEK cells were used as experimental model. Constructs (pAC-N1-GFP) encoding wild-type human CaSR (hCaSR-wt) and its constitutively active (hCaSR-R990G; hCaSR-N124K) and inactive variants (hCaSR-del121), fused with the green fluorescence protein (GFP), were transiently transfected in HEK cells. Immunofluorescence experiments revealed that both hCaSR-wt and the activating variants were expressed at the plasma membrane, whereas the inactive form always localized intracellularly.
Functional studies, with Fura2-AM, indicated that the physiological agonist, calcium (Ca++ 5mM), and the calcimimetic NPS-R568 (5µM) induced a significant increase in cytosolic calcium in cells expressing hCaSR-wt compared to mock cells. Interestingly, stimulation with receptor agonists resulted in a significant increase in intracellular calcium in cells transfected with CaSR active variants with respect to hCaSR-wt. No changes in intracellular calcium were detected in hCaSR-del121 (inactive) expressing cells.
Since a strict controlled calcium gradient between cytosol and endoplasmic reticulum (ER) is crucial for transmission of cellular signals, we first measured the basal level of cytosolic calcium at steady state in cells expressing all CaSR variants.
Of note, we found that the basal intracellular calcium concentration was significantly lower in cells expressing hCaSR-wt and its activating variants compared to mock and hCaSR-del121 cells, which is expected to make cells more sensitive to intracellular calcium modifications in response to CaSR agonists. Accordingly, the bulk of the ER-released calcium resulted significantly higher in cells expressing the activating variants compared with cells expressing either hCaSR-wt or hCaSR-del121. Consistent with these data, Fluorescence Resonance Energy Transfer (FRET) using the ER-targeted Cameleon (D1ER) probe, which detects [Ca++]ER directly, demonstrated a significant higher calcium accumulation in cells expressing the activating CaSR variants.
Since the storage of calcium in the ER is mainly regulated by SERCA, the activity and the expression of this pump were evaluated. Compared to hCaSR-wt expressing cells, SERCA expression level was found significantly increased in cells expressing activating CaSR variants. Consistently, this increase in SERCA expression was paralleled by a strong increase of SERCA activity as assessed by dynamic FRET experiments with D1ER probe. In parallel, we found an inverse correlation with PMCA whose cell surface expression was found significant decreased in hCaSR-R990G and hCaSR-N124K cells. This might assure an optimal ER-cytosol gradient

resulting in basal low cytosolic calcium levels and higher accumulation of calcium in the ER in cells expressing activating CaSR variants.

Together these findings indicate that for the efficiency of calcium signaling system, cells monitor cytosolic and ER calcium levels and regulate in parallel the expression of the SERCA in the ER and the PMCA at the plasma membrane. To our knowledge this is the first demonstration that a complex parallel adaptive feedback can explain the molecular basis of constitutively active variants of the CaSR.

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Motor activity affects adult skeletal muscle re-innervation acting via Trk receptors

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Injured peripheral nerves maintain the capability to regenerate, but the regenerative process is slow and functional recovery is very poor. A faster nerve-regeneration was found using treadmill training programs and a role of BDNF signalling was proposed in explaining this improvement.
Recently, the muscle expression of BDNF mRNA and protein under activity control has been reported, encouraging us to study the effect of chronic treadmill mid-intensity running on adult rat muscle re-innervation and to explore the involvement of BDNF and Trk receptors. After nerve crush, muscle re-innervation was evaluated using intracellular recordings, tension recordings, immunostaining, and Western-blot analyses. An extensive muscle multiple innervation was found in running rats that was fully reversed to control values blocking Trk receptors or interrupting the running activity. An increase of muscle multiple innervation was also found in sedentary rats treated with a selective TrkB receptor agonist. The expression of TrkB receptors by intramuscular axons was demonstrated and an increased muscle expression of BDNF was found in running animals. The increase of muscle multiple innervation correlated with a faster muscle re-innervation we found in running animals. We conclude that when regenerating axons contact muscle cells, muscle activity progressively increases modulating BDNF and possibly other growth factors that acting via Trk receptors, induce axon sprouting to re-innervate skeletal muscle. An important role for muscle activity programs in functional rehabilitation is suggested.

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Slow waves evoked by transcranial magnetic stimulation reflect a cortical downstate

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BACKGROUND and OBJECTIVES
In recent years, an increasing number of studies combined Transcranial Magnetic Stimulation (TMS) and high density Electroencephalography (hd-EEG) in order to evaluate the response of corticothalamic modules to perturbation during loss of consciousness (LOC). Specifically, TMS/hd-EEG measurements showed that during spontaneous (non-REM (NREM) sleep), pharmacologically-induced (anaesthesia) and pathological (severe brain injury) LOC, the thalamocortical system responds to a perturbation producing a positive-negative wave that highly resembles the spontaneous sleep slow waves.
Animal and human studies show that sleep slow waves consist of an alternation between periods of intense firing, called up-states, and periods of neuronal silence, called down-states, associated with a high frequency EEG power decrease.
We hypothesize that also the slow waves evoked by TMS during LOC are characterized by EEG high-frequency suppression (>15Hz), thus reflecting a cortical downstate.
We therefore recorded TMS/EEG responses in 10 healthy subjects during NREM sleep and Midazolam-induced anaesthesia (MA), and in 5 neurological patients in a vegetative state (VS), and compared these responses to 5 healthy awake subjects.
METHODS
TMS was delivered at different cortical locations along the cerebral cortical midline using a biphasic focal coil guided by an infrared navigation system (NBS). Scalp EEG was recorded with a 60-channel TMS compatible amplifier. Time-frequency analysis in all subjects was carried out with customized MATLAB scripts based on Wavelet Transform (Morlet, 3.5 cycles), considering the electrode closest to the stimulation site.
RESULTS
As compared to wakefulness, TMS/EEG responses during all the LOC conditions and for all the subjects were characterized by a large positive-negative deflection. Time-frequency analysis showed a significant suppression of high frequency EEG power (>15 Hz) associated with this slow wave, thus reflecting a cortical downstate.
Interestingly, we also longitudinally monitored 3 VS patients progressively evolving from their clinical condition and eventually regaining consciousness. In these patients, TMS/EEG responses were characterized by a progressive recovery of EEG high frequency, closely matching patients' clinical evolution.
CONCLUSION
Our results show that LOC is characterized by a cortical downstate in response to a direct cortical perturbation, possibly disrupting fast long-range interactions between different thalamocortical modules, a key requirement for consciousness.

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APP-dependent neurogenesis impairment of neural precursors from the Ts65Dn mouse, a model for Down sindrome

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Mental retardation in Down syndrome (DS) appears to be related to severe proliferation impairment during critical phases of brain development. Recent evidence shows that not only cellular proliferation but also phenotype acquisition and dendritic maturation are heavily compromised in DS. In spite of numerous efforts, the mechanisms whereby gene triplication leads to neurogenesis impairment in DS have been poorly elucidated. We recently provided evidences that the trisomic amyloid precursor protein (APP) gene may be a key determinant of neuronal precursor proliferation impairment in DS. We demonstrated that the proliferation impairment of neuronal precursors (NPCs) from the Ts65Dn mouse, a model of DS, was due to derangement of the Shh pathway. We found that trisomic NPCs had an increased expression of the Shh receptor Patched1 (Ptch1), a membrane protein that suppresses the action of a second receptor, Smoothened, thereby maintaining the pathway in a repressed state. We found that Ptch1 over-expression was related to increased levels of the APP intracellular domain (AICD), suggesting that the APP/AICD system underlies derangement of Shh signaling and, consequently, proliferation defects. The overall goal of this study was to determine whether the triplicated gene APP contributes to neurogenesis impairment in DS by influencing, in addition to proliferation, phenotype acquisition and neuronal maturation.
We found that trisomic NPCs showed reduced neuronogensis (acquisition of a neuronal phenotype), increased astrogliogenesis and decreased neuronal maturation (neurite elongation), suggesting that cultures of trisomic NPCs are a suitable model to study the mechanisms underlying neurogenesis impairment in DS. We found that differentiated trisomic NPC cultures, similarly to undifferentiated ones, had an increased expression of APP and Ptch1. Restoration of APP expression fully restored phenotype acquisition and neuronal maturation, indicating that triplicated APP underpinned all aspects of neurogenesis impairment in trisomic NPCs. Shh pathway activation, induced by SAG, and silencing of Ptch1 expression restored neuronogensis and neuronal maturation but not astrogliogenesis in trisomic NPCs. These results suggest that APP-dependent alterations of independent pathways are responsible for the reduction in neuronogenesis and increase in astrogliogenesis, respectively, of trisomic NPCs. Recently, it has been shown that the over-expression of the secreted fragment of APP (sAPP) may result in enhancement of the IL-6 family cytokines, which are crucial for the acquisition of an astrocytic phenotype. We found that blocking of the secreted fragment of APP (sAPP) reduced astrogliogenesis in trisomic NPCs. Moreover, trisomic NPCs exhibited deregulation in the expression of downstream signaling molecules of the IL-6-pathway, suggesting that sAPP increased astrogliogenesis in trisomic NPCs through activation of the IL-6-associated signaling cascade.
Our findings indicate that two different domains of the triplicated gene APP impair neurogenesis in trisomic NPCs. We propose that the APP/AICD system regulates proliferation, neuronogenesis and neuronal maturation, through the Shh pathway, while astrogliogenesis depends on the APP/sAPP system, through an IL-6-associated signaling cascade.

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Molecular mechanisms involved in psychosine-induced apoptosis

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Galactosylsphingosine, also known as psychosine, is an intermediate involved in the catabolism of monoglycosylceramides. It is primarily produced through the galactosylation of sphingosine and is present only in very low levels in normal tissue. A major enzyme responsible for the production of psychosine is ceramide galactosyltransferase (CGT), while its degradation is carried out by β-galactosylceramidase (GALC). Krabbe disease (KD) or globoid cell leukodystrophy (GLD) is an inherited autosomal recessive lysosomal storage disease in which the loss of function of the lysosomal enzyme GALC leads to progressive accumulation of undigested galactosylsphingolipids and severe neurodegeneration characterized by loss of oligodendroglial cells, extensive demyelination in the nervous system and the appearance of distinctive multinucleate globoid cells in the white matter. Tissue from patients with KD are also deficient in enzyme activities which cleave galactose from other glycolipids: galactosylceramide, monogalactosyldiglyceride, and lactosylceramide. Psychosine, that accumulates in the brain of human patients with KD and in several mammalian species including the twitcher mouse, is a highly toxic compound: it may affect different signaling pathways, including activation of phospholipase A2 and upregulation of AP-1 in oligodendrocytes, cell apoptosis, cytokine activation, peroxisomal dysfunction and calcium homeostasis alteration. However, very little is understood about the molecular mechanism by which psychosine induces apoptosis. One recent mechanistic breakthrough is the finding that psychosine accumulates to high levels in lipid rafts (LRs) and that this affects the activity of protein kinase C (PKC). LRs are defined as unique regions of the cell membrane that have a characteristically high concentration of cholesterol and sphingolipids. The abnormal accumulation of psychosine may introduce architectural and functional changes in these domains, leading to cellular dysfunction, brain deterioration and irreversible neurological handicap in the incurable KD. The purposes of this research, that is actually in progress, are: 1) to determine and compare the different apoptotic pathways activated by psychosine on normal and KD cutaneous human fibroblasts as well as on normal (OLP) and twitcher (OLP-TWI) mice oligodendrocytes; 2) to study the associated signals to the progressive psychosine accumulation, that may lead to disruption of LRs.
The first preliminary results indicate that there is much greater sensitivity to psychosine in oligodendrocytes than in fibroblasts. In fact, 50 μM psychosine induced up to 50% and 90% cell death in OLP and OLP-TWI, respectively. Moreover, psychosine treatment resulted in the inhibition of PI3K/Akt pathway related to induction of PTEN expression and downregulation of PI3K. Decrease of the Pdcd4 levels was also detected in psychosine-treated cells, that seems to be linked to iNOS and TRAIL induction in fibroblast and in OLPs, respectively. Probably, the psychosine-promoted JNK pathway, described in literature, inhibits the Pdcd4 expression; the Pdcd4 inhibition leads to an increased AP-1-dependent transcription of TRAIL in glial cells, and an activation of inflammatory stimuli in fibroblasts. Finally, the accumulation of psychosine was accompanied by changes in the distribution of the LR markers flotillin-2 and caveolin-1. However, these findings await confirmation.

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Generation of humanized Dopamine Transporter Knock-in mouse strains carrying loss-of-function mutation as a model of human Dopamine Transporter Deficiency Sindrome

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Dopamine Transporter Deficiency Syndrome (DTDS) is a newly recognized autosomal recessive disorder related to impaired Dopamine Transporter (DAT) function and the first identified neurological disorder caused by genetic alterations of DAT in a cohort of 11 children. Movement disorders such as parkinsonism, hyperkinesia and hypokinesia were described during infancy of these patients. In all cases, homozygous or compound heterozygous SLC6A3 DAT gene mutations were detected. Loss of DAT function was recorded in all missense variants (9 mutations total were found) through in-vitro functional studies.
Humanized DAT (hDAT) knock-in mouse models featuring two particular mutations found in human SLC6A3 gene leading to DTDS phenotype are planned to be generated by means of microinjection of recombinant Embryonic Stem cells (ES) into C57BL/6 mouse blastocysts.
Transfection of ES cells has been performed using plasmid vectors targeted with mouse DNA fragment containing site of mutation, either deletion of Guanine 399 in the DAT coding sequence (CDS) or single base mutation from Thymine to Cytosine at the residue 671 of DAT CDS. Selection markers were inserted in mouse DNA fragment – neomycin resistance gene with its promoter and poly(A) signal and the Thymidine Kinase gene, both flanked by two LoxP sites. Standard diphtheria toxin/G418 double selection protocol was performed to select transfected ES cells. Transient trasfection of ES cells with CRE recombinase expression construct allowed the removal of the LoxP flanked region, and Ganciclovir negative selection was carried out to select clones undergone to homologous recombination of the targeted DNA fragment.
Development and characterization of these Knock-In mice will allow detailed investigation of the pathological molecular mechanisms involved in this disorder and generate experimental test systems for finding new treatments for DTDS and disorders related to DAT dysfunction in general.

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Presynaptic targeting of optogenetic probes

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The optical control of neuronal activity by gene delivery of opsins into neurons constitutes a revolutionary approach to probe the function and connectivity of the neural network. The recent development of optogenetics offers the possibility of high-speed mapping of brain circuitries with high spatial and temporal resolution. Moreover, modulating presynaptic signals with light and detecting postsynaptic responses can help understanding the physiological properties of neuronal circuits. The range of currently available optogenetic constructs still lacks functional presynaptic-targeted opsins. Presynaptic targeting of optogenetic probes is a crucial step to selectively manipulate the presynaptic components of brain circuits, to understand their computational and integrative properties and study the consequences of manipulations on the activity of the entire network. Such targeting can be obtained by fusing the probes to sequences that would mediate their localization to the presynaptic compartment of neurons. Among such sequences, we have employed the synaptosomal-associated protein 25 (SNAP-25). SNAP-25 is a component of the SNARE complex that is mainly located on the cytoplasmic face of the plasma membrane in presynaptic terminals and throughout the axon, where it mediates the process of synaptic vesicle fusion. The SNAP-25 sequence was cloned downstream of the excitatory opsin ChETA, a fast variant of ChR2, and of the inhibitory opsin Arch. In a first set of experiments, the presynaptic targeting was confirmed in primary hippocampal neurons by immunostaining with several pre- and post-synaptic markers for both probes. In a second series of studies, we started to characterize the functional and optical properties of these chimeras, using patch-clamp techniques in autaptic and low density neuronal cultures. Our preliminary results showed an increase of excitatory postsynaptic currents upon illumination of neuronal cultures transfected with presynaptic ChETA. Moreover, we have started to test the localization and the functional properties of our probes in vivo, by lentiviral injection into various areas of the adult brain, in rodents. In some ongoing experiments, we are targeting a number of neuronal subpopulations of the cortico-striatal pathways. The physiological properties of our presynaptic-targeted probes will be studied first ex vivo in brain slices, and subsequently in living animals by performing appropriate behavioral tests.

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Role of hypoxia in the regulation of microvesicles-derived microRNAs in colorectal cancer cells

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Introduction. Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression. Cancer cells release small membrane vesicles (microvesicles) and it has been observed in colorectal cancer (CRC) that microvesicles may influence tumor progression. Microvesicles contain several bioactive molecules such as microRNAs (miRNAs) that are known to regulate several processes, including tumor angiogenesis, which is a potent stimulus for cancer progression. Whether hypoxia, a strong stimulus for tumor angiogenesis, regulates microvesicle-derived miRNAs is still unknown. As a first step of a study aimed at identifying novel mechanisms involved in CRC angiogenesis, we evaluated the role of hypoxia in the regulation of microvesiscles-derived miRNAs. Preliminary experiments were performed to investigate the effect of hypoxia on the expression of microvesicle-derived miRNAs in colorectal cancer cells.
Methods. A thorough literature search was performed to identify genes involved in CRC angiogenesis and five different computational algorithms were used to identify putative miRNA target genes. As it is difficult to judge which algorithm produces the most reliable and/or sensitive target prediction, targets predicted by at least three tools were selected. The culture medium derived from the CRC cell line HCT 116 was used for the isolation of microvesicles. Microvesicles were isolated by ultracentrifugation. Total RNA was extracted from microvesicles and used for miRNA isolation. Using real time RT-PCR we verified the presence of selected miRNAs in microvesicles as well as the effect of hypoxia on miRNA expression.
Results. Ten putative genes were found to be involved in CRC angiogenesis. Using bioinformatic approaches, fourteen novel miRNAs putatively targeting the selected genes were identified. Some of the selected miRNAs, including miR-17, miR-18b and miR-200c were found to be contained in microvesicles and up-regulated by hypoxia. Some other miRNAs, including miR-132, were not expressed in microvesicles.
Conclusions. Our preliminary results demonstrate that the expression of microvesicle-derived miRNAs is influenced by hypoxia in CRC cells suggesting that miRNA deregulation may play a major role in hypoxia-induced CRC angiogenesis. Results from several clinical trials proved that targeting tumor-mediated angiogenesis improves overall survival in CRC patients, although limitations in efficacy, duration and potential toxicity of therapies indicate that further therapeutic approaches are needed. In this respect, the elucidation of the molecular mechanisms underlying CRC angiogenesis may be a key in developing novel therapeutic strategies for CRC treatment. In particular, identifying the role of miRNAs in CRC cells and targeting deregulated miRNAs may be a good approach in preventing CRC angiogenesis.

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Characterization of lysine - containing dipeptides transport by different PepT1 isoforms expressed in *Xenopus laevis* oocytes

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PepT1 (SLC15A1) is an electrogenic transporter that uses an inwardly directed proton electrochemical gradient to drive the transport of di- and tri- peptides and peptido-mimetic molecules into a variety of cells. During digestion, dietary proteins are enzymatically cleaved and degradation products are translocated from the intestinal lumen into the enterocyte via PepT1. Lysine is an essential amino acid which is growth-limiting in fish. The use of alternative protein sources in fish meal causes a reduction of fish growth and feed utilization efficiency and an increase of diseases. Plant protein sources are used to replace fish meal in feed formulations, but are inferior in their essential amino acid (EAA) composition. With increasing use of plant protein sources in fish feeds, EAA supplementation becomes necessary.
PepT1 is the major route of intake of peptides and a better knowledge of the kinetic characteristics of the transporter related to lysine containing peptides will help to add EAA in the correct form.
Aim of this work was to explore the transport of different combinations of lysine, methionine and glycine dipeptides, in order to evaluate the transport efficiency using electrophysiological methods in various PepT1 isoforms.
Measurements of the transport associated currents generated by the transporters, in the presence of different substrates, were performed at constant or different membrane voltages and at two pH conditions by the classical two-electrode voltage-clamp technique on *Xenopus laevis* oocytes expressing sbPepT1(*Dicentrarchus labrax*) rbPepT1(*Oryctolagus cuniculus*) and zfPepT1 (Danio rerio).
The transport currents, the I/V relationships, the dose-response analysis were determined and the kinetic parameters as the maximal current (*Imax*), the substrates apparent affinity ($K_{0.5}$) and the transport efficiency ($Imax / K_{0.5}$) were calculated.
Species-specific differences were observed in the potency order among the various substrates (tested at 1 mM), and in the voltage-dependence of the current amplitude. Particularly Lys-Met was the best substrate at all tested potentials in seabass PepT1, as well as in the rabbit transporter, while in the zebrafish isoform all tested dipeptides (except Gly-Lys) elicited similar currents independently of the charge position or amino acid composition. In all isoforms the substrate potency order was pH-independent in the range 6.5 -7.5, and the tripeptide Lys-Lys-Lys did not give rise to any current. In rabbit and seabass PepT1s the dipeptide Lys-Lys was only modestly transported at all voltages, while in the zebrafish a strong current increase was observed at negative membrane potentials. The dose response curves were determined at different voltages from -140 to -20 mV and the ratio of the relative $Imax/K_{0.5}$ was calculated. In the seabass and rabbit PepT1 these parameters show the importance of the position of lysine in the dipeptide. These proteins have very low affinity for Lys-Lys and Gly-Lys that highly reduces the transport efficiency; the other dipeptides tested were quite similar in affinity (between 0.2-0.8 mM) with small changes with voltage, but the transport efficiency was significantly higher for Lys-Met and Lys-Gly. In zfPepT1 relatively high affinity and excellent transport efficiency were shown by Met-Lys and Lys-Met, while low efficiency was found for Gly-Lys. The physiological and nutritional relevance of these results can suggest the possible forms of EAA supplementation.

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Do salivary proteins influence bitter taste to 6-n-propylthiouracil (PROP) in humans?

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The genetic predisposition to taste 6-n-propylthiouracil (PROP) varies greatly among individuals and may be correlated with chemical composition of saliva. We investigated the possible relationship between PROP bitter taste responsiveness and salivary proteome. In addition, in order to understand the role that salivary proteins could play in the PROP perception, we analyzed effects of supplementation of the specific basic proline-rich proteins (bPRP) on PROP responsiveness.
Sixty-two subjects (21 males, 41 females, age 25 ± 3 y) were classified for their PROP taster status by rating their taste perception intensity evoked by PROP and NaCl solutions. Salivary protein quantitative and qualitative determination was performed by HPLC-ESI-MS analysis in individuals classified by PROP status before and after PROP taste stimulation. PROP responsiveness was assessed before and after Ps-1and II-2 supplementation in individuals lacking them.
Subjects were classified as PROP super-tasters (n = 24), medium tasters (n = 17) or non-tasters (n = 21). Post-hoc comparisons subsequent two-way ANOVA showed that only basal levels of II-2 and Ps-1 proteins of the bPRPs family were significantly higher in un-stimulated saliva of super-tasters and medium tasters than in non-tasters. Pairwise comparisons subsequent to three-way ANOVA showed that PROP stimulation determined an increase of the II-2 and Ps-1 protein levels of super-taster saliva with respect to the basal levels. In addition, preliminary data showed that PROP bitterness of individuals lacking Ps-1and II-2 increased significantly after supplementation of these proteins.
Our results show for the first time that responsiveness to PROP is strongly associated with proteome, and suggest that the II-2 and Ps-1 proteins could interact by carrying the stimulus to receptor sites, as transporters of hydrophobic molecules.

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Statins treatment increases AQP2 plasma membrane expression in vitro and in vivo: potential usefulness in the treatment of nephrogenic diabetes insipidus (NDI)

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The water channel Aquaporin 2 (AQP2) is responsible for the vasopressin (VP)-dependent water reabsorption occurring in the kidney during antidiuresis.
X-linked nephrogenic diabetes insipidus (XNDI), a severe rare disease characterized by impaired urine-concentrating ability of the kidney, is caused by inactivating mutations in the V2 type VP receptor (V2R) gene. Mutation prevents the VP-induced shuttling of AQP2 from intracellular storage vesicles to the apical plasma membrane of kidney collecting duct principal cells. This, in turn, dramatically reduces water reabsorption, resulting in severe polyuria and constant risk of dehydration. Unfortunately, the current pharmacological approach for handling XNDI is unable to rescue AQP2 membrane expression.
We have previously reported that the cholesterol-lowering drug lovastatin increases AQP2 membrane expression in renal cells in vitro.
More recently we reported that, in mice, fluvastatin increases AQP2 membrane expression in the collecting duct in a VP-independent fashion and greatly increases the amount of water reabsorbed in the kidney.
Additional experiments in vitro, performed on a cell culture model recapitulating AQP2 trafficking, indicate that this effect of fluvastatin is most likely caused by the statin-dependent inhibition of protein prenylation of key regulators of AQP2 trafficking in collecting duct cells. We identified members of the Rho and Rab families of proteins as possible key players whose reduced prenylation might result in the accumulation of AQP2 at the plasma membrane, by modulating the basal rate of exocytosis and/or endocytosis.
Most importantly, preliminary results obtained using the conditional mouse model of human XNDI, characterized by severe polyuria and low urine osmolality, indicate that fluvastatin treatment significantly reduces diuresis and increases urine osmolality.
Taken together, these results strongly suggest that statins may prove useful in the therapy of XNDI.

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Gender differences in maximal strength improvement and muscle fiber characteristics after 8 weeks of resistance training

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Introduction
It is well know that heavy resistance training (RT) promote skeletal muscle hypertrophy (Andersen &Aagaard 2010). In particularly some studies have shown that this kind of training tends to decrease the expression of MHC IIx and MHC I and, at the same time, increase the expression of MHC IIa. But gender differences in such kind of changes are not known. The aim of this study was to analyse the differences in gender response after two months of RT. Furthermore, we wanted to compare the changes in latissimus dorsi muscle fibres characteristics with the mechanical measurement of fibres.
Methods
Eighteen healthy volunteers participated in 8-week progressive resistance training for upper limbs muscles. One repetition maximal test was performed and mechanical and myosin characterization (Pietrangelo et al. 2009) of latissimus dorsi muscle fibres were analysed pre and post- training. We used a fine needle biopsy technique that allowed us to obtain about 4 mg of muscle sample (Paoli et al. 2010).
Results
The increase in 1RM after 8-weeks of training was significantly greater in women (+24%) compared to men (+13%). The electrophoretic analysis of muscle fibers showed some changes in MHC expression. We observed an increased in MHC IIa (male +13% and female +33%) and a decrease in MHC IIx (male -8% and female -26%) while the MHC I expression in male tends to decrease (-5%) and in female tends to increase (+6%). Interestingly, the two-way ANOVA analysis (time x gender) showed a gender significant difference for female only in MHC IIa expression.
The mechanical analysis of single fibres showed that training increased significantly the cross sectional area (CSA) and fibre strength in male and muscle fibre tension both in male and female.
Discussion
The greater increase of 1RM performance with a substantial unchanged fibre CSA in female subjects could be explained by an improved motor units recruitment whilst males response to training seems oriented to hypertrophy. Moreover also myosin changes showed a gender related difference. Taken together our results suggest that resistance training effects on muscle are gender specific and more work is needed to explain this difference.

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Transient increase in neuronal chloride concentration by soluble factors released from glioma cells

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I frequent a first year of PhD school in Neurophysiology. I conduct electrophysiological study on microglial activation and apply imaging technique on study of neuronal physiological alteration induced by glioma cells.
Gliomas are the most common primary tumor in the central nervous system (CNS). Epilepsy is frequent in patients with brain tumors; several processes are involved in glioma-related seizures, including a reduced γ-aminobutyric acid (GABA)ergic inhibition and an altered chloride homeostasis. However, the mechanism leading to changes in $[Cl^-]_i$ in neurons during glioma invasion are still unclear.
In the current study, we investigated, with an in vitro approach, the alteration of intracellular chloride concentration, in hippocampal neurons, induced by substances released from glioma cells. To do this, we employed an imaging technique, using a genetically encoded CFP/YFP-based Cl-Sensor transiently expressed in cultured hippocampal neurons, that allows non-invasive ratiometric estimation of $[Cl^-]_i$ using fluorescence monitoring [Markova, 2008; Bregestovski, 2009; Waseem, 2010].
The application of glioma conditioned solution (GCS) to neurons (10-12 DIV) transfected with Cl-Sensor induced a rapid and transient elevation of $[Cl^-]_i$; this resulted in the increase of fluorescence ratio (R) from 0.51±0.01 to 1.03±0.05 (n=63).
The magnitude of the GCS-induced Cl^- response was considerably reduced by the pretreatment of neurons with NPPB (100 μM), a non-specific chloride channels blocker, and furosemide a blocker of both NKCC1 and KCC2, indicating that both the anion channels and Cl^- cotransporters are a major routes for glioma-induced Cl^- influx.
We then tried to identify which substances released by glioma cells could trigger CGS-induced Cl- increase; responses to GCS were strongly reduced in presence of ionotropic glutamate receptor antagonists, APV (20 μM) and NBQX (10 μM); moreover, similar magnitude of the GCS-induced responses were observed following application of 100 μM glutamate (from 0.55±0.02 to 1.01±0.08, n=28). These data indicate that the activation of ionotropic glutamatergic receptor is necessary to GCS-induced Cl^- increase, suggesting that glutamate released by glioma cells is involved.
We, then, tested the involvement of $GABA_A$ receptor, since GABAergic receptors are among the major ways of access for chloride in neurons, using the antagonist bicuculline (10 μM); unexpectedly, the magnitude of the GCS-induced Cl^- responses was enhanced by bicuculline (ΔR=1.61±0.34) compared with control in the same experiments (ΔR= 0.19±0.06).
These data led to the conclusion that GCS contains both glutamatergic and GABAergic agonists. The GCS induced increase of chloride concentration is likely triggered by glutamate-induced depolarization and contrasted by GABA induced hyperpolarization or shunting. This was confirmed by current clamp experiments, where CGS-induced depolarization of neurons was enhanced by bicuculline and abolished by (APV/NBQX).

Altogether, our results demonstrate the effectiveness of Cl-Sensor for non-invasive monitoring of $[Cl^-]_i$ and suggest that glioma cells might affect Cl^- homeostasis in neurons through a glutamatergic mechanism, which is expected to deeply influence neuronal network excitability.

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Synapsin function in the regulation of the synaptic vesicle cycle in presynaptic terminals of hippocampal neurons

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Besides the well known synapsin (syn) role in the control of synaptic vesicle (SV) trafficking between the reserve pool and the readily-releasable pool of SVs through phosphorylation-dependent interactions with actin and SVs; recent data have suggested that syn can play novel functions in other aspects of the pre-synaptic physiology, such as SV docking, fusion and recycling (Bloom et al., 2003; Akbergenova and Bykhovskaia, 2010; Coleman and Bykhovskaia. 2010; Orenbuch et al., 2012). Clues of a role of syn in SV recycling came from the fact that syn, localized in the vesicle cluster at rest, transiently accumulates at endocytic sites after intense stimulation, where it interacts with nascent recycling vesicles (Bloom et al., 2003). Moreover several dynamin-binding proteins involved in SV endocytosis including syndapin (Kessel and Qualmann 2004), amphiphysin and endophilin all bind synapsin.
The aim of this project is to dissect syn contribution to the regulation of the SV cycle in presynaptic terminals of hippocampal neurons. The possible role of syn in SV retrieval and in the spatial organization of recently endocytosed SVs was studied. This was done by studying endocytic recycling in primary cultures of hippocampal neurons derived from wild type (WT) mice and syn triple knock-out (TKO) mice by means of pHluorin assays, immunofluorescence and transmission electron microscopy.
Synaptophysin-pHluorin, a pH-sensitive used to investigate SV recycling dynamics, showed no major difference in terms of endocytic kinetics (tau of endocytosis) between WT and TKO at various frequencies of electrical stimulations.
At the ultrastructural level, under resting conditions, or in nerve terminals subjected to an electrical stimulation (300 action potentials (AP) at 10 Hz) the number of clathrin-coated structures were comparable in WT and syn TKO synapses. Also, the number of endosomal structures at rest or right after stimulation was similar in both genotypes. However while in WT neurons the number of endosomal structures decreased rapidly after stimulation, endosomal structures were present for longer time in the TKO, suggesting a possible defect in SV sorting from endosomal intermediates.
To investigate the origin of these endosomal structures, cultured neurons were electrically stimulated in the presence of soluble horse-radish peroxidase (HRP), an endocytic marker that internalizes upon SV endocytosis and locally catalyzes a peroxidation reaction that mediates the formation of an electron-dense substrate.
The HRP-uptake assay did not show any appreciable difference in the amount of SVs recycled after the stimulus. On the contrary, the “mild” stimulation procedure also produced the formation of few HRP labeled endosomes. Although the number of these structures was highly variable, TKO terminals showed a delayed kinetics in the processing of HRP-labeled endosomes.
Although syn interacts with the clathrin mediated endocytosis (CME) machinery, our data did not suggest a major role in SV retrieval. Our data, instead, point to a possible role of syn in regulating SV cycling to/from the endosomal intermediates. It is tempting to speculate that syn might have a role in the formation of vesicles from recycling endosomes inside presynaptic terminals also in light

of the fact that syn has been reported to sense membrane curvature by an amphipathic lipid packing sensor motif (Krabben et al., 2011).

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Does a different susceptibility to endocrine disruptors between sexes exist? The example of VSMC motility

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Sex steroid hormones modulate the phenotype and function of many target cells both with rapid and slow mechanisms. In fact, estrogens and androgens are not only involved in the regulation of the reproductive function, but they have diverse and prominent effects on all male and female physiology including regulation of cardiovascular, nervous, and muscle systems. As a consequence the incidence of pathologies could also differ between sexes. In particular, cardiovascular disease is less prevalent in pre-menopausal women than in men, but its occurrence in women increases at the onset of menopause; moreover, the loss of female sex hormones contributes to the striking increase in the incidence of cardiovascular morbidity and mortality in postmenopausal women. Vascular smooth muscle cells (VSMC) motility is a normal process that occurs during vascular development or for tissue repair in response to vascular injury. At the same time, pathological migration is a major factor in atherogenesis and restenosis. Sex steroid hormones differently impact on VSMC migration. It has been demonstrated that 17β-estradiol (E2) impairs proliferation and migration of human vascular smooth muscle cells induced by growth factors, whereas few data are available on the effects of androgens on VSMC. Differences in the levels of both estrogen receptors (ERα and ERβ) and androgen receptor (AR) are known in VSMCs isolated from male and female rats. In particular, the ERβ subtype level shows a clear gender difference, being lower in VSMCs from female than in cells derived from male. It results in a significant gender difference in the ERα/ERβ ratio that is completely abolished in VSMCs at 14th passage.
Endocrine disruptors (EDs) are natural and man-made exogenous substances present in the environment which can interfere with permanent and activational effects of sex steroid hormones by binding to their receptors, impairing male and female physiology. EDs interferences have been reported in many physiological and pathological states, such as: reproductive system, immune function, bone structure, adipose tissue, nervous system, cancer onset and cardiovascular function.
The aim of this work was to study the gender-related molecular mechanisms of sex steroid hormone-induced VSMCs motility and the impact of EDs in mechanisms. In particular, naringenin (Nar, a plant-derived flavonoid) and bisphenol A (BPA, a synthetic placistiser) impact has been investigated in VSMCs derived from male and female rats.
Our data show that E2, but not testosterone and dihydrotestosterone, is able to reduce the VSMC motility via the ERβ-dependent rapid activation of p38/MAPK. Nar acts as an ERβ agonist mimicking the hormone effects, while BPA acts as a pure E2 function antagonist preventing the E2-induced signals preventing the E2 effects. Intriguingly, Nar stimulation reduces cell motility also in male VSMC both in the absence and in presence of androgen hormones. This effect requires ERβ which is expressed in male cells. All together, these results confirms the protective role of estradiol on cardiovascular system and highlights that the estrogen signaling is more prone to ED interference than androgen signaling. However, male and female express all steroid sex hormone receptors (i.e., ERs and AR) and thus they result equally susceptible to endocrine disruption. Differences could arise in dependence on timing of exposure to EDs and target tissues.

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Neuronal downstates and cortical breakdown of causality during NREM sleep: an intracerebral study in humans

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BACKGROUND and OBJECTIVES
Recent studies have combined Transcranial Magnetic Stimulation (TMS) and high density Electroencephalography (hd-EEG) to evaluate how cortico-cortical information transmission changes upon falling asleep. These experiments show that, as opposed to wakefulness (W), EEG responses evoked by TMS during non-REM (NREM) sleep are characterized by positive-negative waves closely resembling spontaneous sleep slow waves (<4Hz). We hypothesized that slow waves triggered by a direct external perturbation during NREM are due to the bistability between neuronal up- and down-states in thalamo-cortical circuits. This hypothesis rests on animal and human studies showing that both spontaneous EEG sleep slow waves and K-complexes are associated with strong and consistent decreases in EEG-gamma (>20Hz) and multiunit activity reflecting a cortical downstate. In the present study, we perform intracerebral electrical stimulation (ICS) and simultaneous intracerebral recording in human to test the hypothesis that during NREM (i.e. a physiological model of loss of consciousness) also an external perturbation (here ICS) can induce a cortical downstate (due to bistability) correlating with the occurrence of a cortical breakdown of causality. To this aim we compare cortico-cortical evoked potentials (CCEPs) evoked during W and NREM.

METHODS
Eight patients with drug resistant epilepsy implanted with multi-channels depth electrodes (Nihon Kohden) were recorded for clinical evaluation. The number of implanted electrodes varied based on surgical requirements (max: 15 electrodes, 168 channels). During W and SWS, stimulation trains (30 shocks, 1Hz, 5mA) were delivered through one single channel, while recordings of CCEPs were obtained from all other channels. Preprocessing and data analysis, consisting in time-frequency analysis and phase locking measures, were performed using customized MATLAB scripts (MathWorks).

RESULTS
During wakefulness, ICS evoked a sustained response made of recurrent waves of activity showing different components (>4Hz) persisting until 300 - 500 ms. Conversely, during NREM sleep ICS evoked one high amplitude and long lasting (300 - 600 ms) slow wave (<4Hz). After this, no further stimulus-locked activity could be detected. Time-frequency analysis showed that: (i) low frequency

power evoked by ICS was significantly higher during NREM then in W; (ii) during NREM EEG-gamma activity (>20Hz) decreased significantly in correspondence to slow waves evoked by ICS thus reflecting a cortical downstate. Moreover, phase locking measures showed that suppression of high frequency correlates with a breakdown of synchronization both within a given channel (Phase Locking Factor) and between different channels (Phase Locking Value) reflecting a breakdown of causality.

CONCLUSIONS

These results suggest that, during NREM, when consciousness fades, the occurrence of a cortical down state after an initial neuronal activation (up-state, both spontaneous or induced) impairs the ability of thalamocortical circuits to mutually reiterate information, generating complex responses, a theoretical requirement for consciousness.

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Genetic methods to manipulate brain aging in the short-lived fish Nothobranchius furzeri: an novel model species for aging studies

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Aging can be considered a physiological status of mental and physical decay continued over time. The decrease in neurogenesis and the general deterioration of the nervous system are closely related to the onset of neurodegenerative diseases, which, in light of the increasing median age of the population, represent a social challange. Investigations into vertebrate aging are hampered by the lifespan of the available model systems. The annual fish Nothobranchius furzeri is the vertebrate with the shortest lifespan and is emerging as an alternative model species. This species has a life expectancy of just 6-7 months and presents the typical features of aging observed in mammals (Genade et al., 2005), including gliosis and reduced neurogenesis (Terzibasi Tozzini et al., 2012). This species can be easily used to assess the effects of environmental interventions on aging parameters (Terzibasi et al., 2009; Valenzano et al., 2006). Aim of the following project is to develop techniques to allow genetic manipulations in the brain of N.furzeri. MicroRNAs (miRNAs) are small non-coding RNAs and have emerged as new key players in the regulation of gene expression. By binding to their target mRNAs, they influence its stability and translation, thereby acting as a new level of regulation which follows transcriptional regulation. We identified a number of evolutionarily-conserved miRNAs that are regulated during aging in N. furzeri (Baumgart et al., 2012). In particular, we focus on mir-29a and the cluster miR 17~92: the former is associated with cell senescence and is up-regulated upon aging, the latter is involved in cell proliferation and is down-regulated upon aging. We created expression plasmids to overexpress these microRNAs in vivo, we then:

1. Demonstrated that somatic gene transfer into adult neuronal stem cells is possible in the adult brain of N.furzeri by means of in vivo lipofection.
2. Set-up a protocol of eggs microinjection and observed mosaic integration in the P0 generation. This sets the basis to generate stable transgenic lines in N. furzeri.
3. Compared the expression patterns driven by three short promoters: Hu C/D, miR-124, and ubiquitin in order to select pan-neuronal and ubiquitous promoters for generation of transgenic N. furzeri.
4. Devised a system for inducible overexpression based on the inducible form of the CRE recombinase.

We will use lipofection to overexpress microRNAs directly into the neurogenic niches in young and old fish in order to assess their influence on the life history of adult neuronal stem cells.
In a longer perspective, we aim to over-express microRNAs or their competitive inhibitors (sponges), over different ages of the fish in order to test their role in replication and neuronal differentiation and analyze senescence and neurodegeneration.
The results of this study will highlight some roles of microRNAs in brain aging in N. furzeri. Since regulation of miRNAs during aging is much more conserved in vertebrates than regulation of protein-coding genes, we expect that the results we will obtain in N. furzeri will be of relevance for human brain aging.

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Oxidative stress of respiratory cells induced by H2O2 or cigarette smoke extract is reduced by the subministration of S-CMC-Lys

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The glutathione system has a key role in redox regulatory mechanisms. We have already demonstrated that the mucoactive drug S-carbocysteine lysine salt (S-CMC-Lys) is able to significantly increase glutathione (GSH) secretion in human respiratory cells. In this study we evaluated S-CMC-Lys effects on GSH intracellular content and metabolism after an oxidative stress induced by H2O2 or after the exposure to Cigarette Smoke Extract (CSE).
GSH and Reactive Oxygen Species (ROS) were evaluated with fluorimetric assays in respiratory cell lines 16HBE14o- and WI-26VA4. The expression of GSH related enzymes was detected by real-time PCR.
S-CMC-Lys (100 µM) induces in both cell lines a significant increase of the GSH intracellular content and a significant increase in the expression of the catalytic subunit of γ-GCS (γ-Glutamyl Cysteine Synthase), a key enzyme for the synthesis of GSH. To investigate the role of S-CMC-Lys in oxidative stress, we exposed our cell models to H2O2 (1 or 10 µM) or CSE.
S-CMC-Lys pre-treatment of WI-26VA4 cells prior H2O2 exposure reduced ROS, without affecting the GSH content. Similar results were obtained by pre-treating with S-CMC-Lys the respiratory 16HBE14o- cell line before 5% CSE exposure. At lower CSE concentrations (2.5%)the pre-treatment with S-CMC-Lys, followed by co-treatment with CSE was able to significantly reduce the GSH dropdown induced by CSE. Co-subministration of S-CMC-Lys with 5% CSE for a prolonged period (24 hours) resulted in an increase in the GSH content and a significantly increased level of γ-GCS and GR (glutathione reductase) mRNA, when compared to the sole CSE exposure.
S-CMC-Lys increases the GSH intracellular content of respiratory cell lines, possibly by enhancing the expression of γ-GCS catalytic subunit. The pre-treatment of respiratory cells with S-CMC-Lys may exert a protective function during oxidative stress, thus reducing the ROS-mediated inflammatory response.
The subministration of S-CMC-Lys, by potentiating the adaptive response of respiratory cells to CSE, may be of help in counteracting chronic obstructive pulmonary disease and CSE negative effects on the GSH system and ROS production.

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The regulation of emotional aspects of behaviour: a new role for cholesterol biosynthetic pathway in the central nervous system

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The cholesterol biosynthetic pathway is crucial for most eukaryotic cells. The key and rate-limiting enzyme of this metabolic pathway, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), is retained to play a pivotal role in brain physiology since it leads to the production of isoprenoid compounds, besides cholesterol, that are vital for several neuronal processes. Although clinical evidences suggest a close link between the inhibition of cholesterol biosynthetic pathway and the modulation of brain functions, studies on the behavioral effects are currently limited and controversial, and the molecular mechanisms are poorly understood. In particular, mood changes and depressive syndromes has been observed in patients undergone to hypocholesterolemic treatment. In this study, we investigated the role of cholesterol biosynthetic pathway in the modulation of emotional reactivity in rats. For this purpose, three-month old male Wistar rats were chronically treated (3 weeks) with the HMGR inhibitor simvastatin (1.5 mg/kg die) and tested in social interaction test and elevated plus-maze task in order to evaluate the behavioral effects induced by HMGR inhibition. The results show that in the elevated plus-maze task no significant effects were detected in the standard measures of entries onto and time spent in the aversive open arms between simvastatin-treated and control rats. In contrast, HMGR inhibition by simvastatin induced a significant decrease in the frequency and the time spent in the active social interaction. These data indicate that HMGR inhibition had a selective action on the modulation of emotionality, by specifically inducing the occurrence of a social anxiety-related behavior. The observed effects were mediated by the lack of isoprenylation of the small GTPase rab3, which is deeply involved in the synaptic vesicle release, in both hippocampus and prefrontal cortex. Furthermore, an “in vitro” degradation assay showed that unprenylated rab3 is more susceptible to degradational events in the same brain areas.
Taken together our data demonstrate that HMGR is a crucial enzyme for the regulation of behavioral processes and, more intriguingly, these effects seem to be determined by rab3 activity on neurotransmitter release in specific brain regions. It is concluded that the modulation of HMGR and/or its specific end-products could be considered as prospective molecular target for pharmacological therapies, thus opening a new scenario in the treatment of some psychopathologies.

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Serotonin receptor 7 (5-Htr7): a key-regulator of neurite outgrowth in telencephalic neurons

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The 5-Htr7 is a seven-transmembrane G-protein-coupled receptor, positively linked to adenylate cyclase through the stimulatory Gs protein. Its prominent downstream effectors are protein kinase A (PKA), the extracellular signal-regulated kinases (ERKs) and small GTPases of the Rho family, involved in cytoskeleton rearrangements. This receptor plays a key role in many physiological functions, such as circadian rhythms, body temperature and endocrine regulation, hippocampus-dependent contexual learning and memory processing, and mood regulation. The 5-Htr7 seems also critically involved in human psychiatric and neurological disorders (Matthys et al., 2010).
We have previously shown that in adolescent rodents 5-HTR7 plays a major role in the modulation of self-control behavior, and may subserve the persistent structural rearrangements of the brain reward pathways occuring during postnatal development, following chronic methyphenidate exposure (Leo et al., 2009).
Here we report that there is a direct relationship between 5-Htr7 stimulation and neuronal morphological alterations, at least in vitro. Indeed, treatment of striatal and cortical primary cultures, generated from mouse and rat embryos, with 5-Htr7 agonists (8-OH-DPAT and LP211) induces a significant increase of the neurite le-III□ngth, evaluated by tubulin immunostaining, when compared to untreated cultures. This effect is abolished by pre-treatment of the cultures with a selective 5-Htr7 antagonist, with the ERK inhibitor U0126, with the Cdk5 inhibitor roscovitine, or with cycloheximide, an inhibitor of protein biosynthesis.
Preliminary Western blot analyses coupled to two-dimensional gel electrophoresis reveals both qualitative and quantitative expression changes in selected cytoskeletal proteins, following treatment of striatal primary cultures with LP211. In particular, a strong increase in the expression of the 34 KDa isoform of MAP1B is observed in stimulated cultures, consistent with a role of this protein in tubulin polymerization and neurite elongation.
Our data show that agonist-dependent activation of the 5-Htr7 in CNS neuronal primary cultures stimulates ERK- and Cdk5 -dependent neurite outgrowth, sustained by expression changes of selected cytoskeletal proteins. These data are consistent with the hypothesis that the 5-Htr7 might exert a crucial role in the regulation of neuronal morphology and structural organization of behaviorally relevant neuronal networks during sensitive developmental stages.

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Dendritic pathology and connectivity can be rescued by pharmacotherapy with fluoxetine in the Ts65Dn mouse model of Down sindrome

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Down syndrome (DS) is a genetic disease caused by an extra copy of chromosome 21 and is characterized by severe cognitive impairment, the most invalidating feature of this pathology. Though defective neurogenesis is a crucial determinant of mental disability, a severe dendritic pathology most likely plays an equally important role. In a previous study, using the Ts65Dn mouse model for DS, we found that early pharmacotherapy with fluoxetine, a selective serotonin reuptake inhibitor, fully restored neurogenesis. Since fluoxetine favors dendritic development in normal animals, the goal of our study was to establish whether fluoxetine restores dendritic maturation and connectivity, in addition to neurogenesis. Euploid and Ts65Dn mice were treated with fluoxetine in the postnatal period P3-P15, and killed at 45 days of age. In Golgi-stained brains we reconstructed the dendritic arbor of the granule neurons of the dentate gyrus (DG) with a dedicated software and, additionally, examined spine density. We found that Ts65Dn mice had a severely hypotrophic dendritic arbor and notably fewer spines than euploid mice. Treatment with fluoxetine fully restored both these defects. Consistently with the dendritic hypotrophy and paucity of spines of trisomic granule cells, synaptophysin (SYN) immunoreactivity revealed an overall reduction of the inputs to the molecular layer that was completely restored by treatment. In view of the key role of the glutamatergic innervation on hippocampus-dependent-memory functions, we examined glutamatergic terminals in DG by co-localization analysis of SYN and glutamate vesicular trasporter 1 (VGLUT1). We found that co-localization of SYN and VGLUT1 was reduced in untreated Ts65Dn mice as compared to euploid mice in all zones of molecular layer and treatment with fluoxetine fully restored the glutamatergic input. These findings show that fluoxetine has an impact on the two major defects of the DS brain: neurogenesis failure and dendritic pathology, indicating that the same treatment is able to rescue not only the number of granule neurons but also their "quality", in terms of correct maturation and connectivity. These findings strongly suggest that fluoxetine may be a drug of choice for the improvement of the major defects in the DS brain and, possibly, of mental retardation.

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Electrophysiological evidence for potassium accumulation between type I hair cells and calyx terminals in mammalian crista ampullaris

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Two types of hair cells are present in mammalian vestibular sensory epithelia, called Type I and Type II hair cells, which differ in electrophysiological properties and innervation. Type II hair cells are contacted by several bouton nerve terminals, while Type I hair cells are contacted by a calyx nerve terminal that envelopes the entire basolateral membrane. Only Type I hair cells, moreover, express a low-voltage activated outward K+ current, called IK,L, which confers upon them a much lower input resistance at rest compared to Type II hair cells. As a consequence, in Type I hair cells large transducer currents would be necessary to change the cell membrane potential and to depolarize the cell enough to activate voltage-gated Ca2+ channels and related neurotransmitter release. How the calyx synapse operates remains in fact enigmatic. It has been speculated that K+ accumulation in the synaptic cleft cooperates with conventional (vesicular) synaptic transmission in sustaining afferent transmission by Type I hair cells. By combining the patch-clamp whole-cell configuration with the whole crista preparation, we have recorded the current and voltage responses of mouse semicircular canal Type I and Type II hair cells in situ. Depolarizing voltage steps elicited in Type II hair cells a large outward K+ current characterized by a substantial time-dependent inactivation, while the same voltage-protocol elicited in most Type I hair cells a large and sustained outward K+ current. However, in a notable percentage (51%) of Type I hair cells investigated, the outward K+ current showed a substantial time-dependent relaxation. In these cells, moreover, upon repolarization to –40 mV the instantaneous current was inward, reversing to outward slowly with time. A reasonable explanation for the above results is that during large outward K+ currents, K+ accumulates around Type I hair cells, thus shifting the K+ reversal potential (VrevK+) toward more positive values. The rightward shift of VrevK+ would produce both the outward current relaxation during depolarizing voltage steps and the instantaneous inward current upon repolarization at –40 mV. Since we never observed such effects when recording from Type II hair cells, we hypothesized that the presence of a residual nerve calyx was responsible for K+ accumulation around Type I hair cells. We also found that by using voltage protocols that increased extracellular K+ accumulation, IK,L deactivation was slowed down. Similar results, i.e. VrevK+ rightward shift and IK,L deactivation slowdown, were obtained by local perfusion of the preparation with an extracellular solution enriched in K+, thus corroborating our hypothesis about K+ accumulation.
In conclusion, our results provide electrophysiological evidence for an increased K+ concentration in the synaptic cleft between Type I hair cell and its calyx ending during outward K+ current activation. The resulting depolarization might be aimed at reinforcing and prolonging Ca2+ channels activation and thus afferent transmission during slow head movements detected by vestibular organs.

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NKCC2 trafficking and activity: the role of interacting proteins

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The Na^+-K^+-$2Cl^-$ cotrasporter type 2 (NKCC2) is exclusively expressed in the apical membrane of the thick ascending limb (TAL) in the kidney where is involved in the vectorial transepithelial salt reabsorption. We focused our research on the identification of NKCC2-interacting proteins possibly involved in the trafficking of NKCC2.
Using NKCC2 stably transfected LLCP-K cells we identified by an antibody shift assay, moesin, a protein belonging to ERM family, as an NKCC2-interacting regulatory protein and as a crucial player in the insertion of NKCC2 in the apical membrane. It has been reported that perturbation of NKCC2 membrane expression and activity might have a crucial role in the onset and maintenance of Na-sensitive hypertension. Indeed, we tried to identify the NKCC2 regulatory partners in spontaneously hypertensive rats (SHR) to shed light on the role of NKCC2 in the pathogenesis of hypertension. Using a proteomic approach we found that the NKCC2-containing macromolecular complexes in SHR rat TAL membrane preparations were different from that observed in age-matched control animals suggesting a different supramolecular arrangement of NKCC2 in the membranes of SHR rat kidneys. Taken together, these findings highlight the importance of investigating the dynamics of NKCC2 membrane expression in order to gain insights on the regulation of NKCC2 mediated Na^+ and Cl^- absorption in the kidney in physiopathological conditons.

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A neurogenetic approach to the study of non spatial attention in mice and rats

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Spatial attention of non selective (NSA) and selective (SSA) type can be studied in genetic model systems (neurogenetic approach). This research project has two main aims, i.e. the analysis of two forms of rearings: free rearings or leanings against the walls in various mouse strains or rat lines. The latter are thought to feature different aspects of Attention-Deficit Hyperactivity Disorder (ADHD). The neurogenetic approach was carried out in mice knock-out for Dopamine (DA) -D2 Receptor (R) or D3R homozygous (-/-), heterozygous (+/-) or wild-type (+/+) obtained from Daniela Vallone Institute of Genetics Molecular Biology, CNRS/INSERM/ULP, Strasbourg, F. Conversely, it was carried out in rats of the Naples High-Excitability (NHE) with Random-Bred (NRB) controls; Spontaneously Hypertensive (SHR) with Wistar-Kyoto (WKY) as controls; WK-Hyperactive (WK-HA) and WK- Hypertensive (WK-HT) obtained from Edith Hendley Univ. Vermonth, Burlington, VT. The experimental design included young adult male mice and rats that were tested in a spatial novelty, consisting in a Làt-maze. Frequency of corner crossing (HA), frequency an duration of rearings and leanings were analyzed. Both durations monitor NSA. Results showed in mice difference only for leanings: (i) DA-D2R(-/-) had reduced frequency; (ii) DA-D3R(+/-) had increased frequency; (iii) DA-D2D3R(-/-) and DA-D2D3R(+/-) had reduced and increased frequency, respectively. Rats studies showed: (i) in NHE higher frequency and lower duration of leanings than in NRB, (ii) in SHR higher frequency of rearing and leanings than in WKY, (iii) in WK-HA higher frequency of rearing and leanings and higher duration of leanings than in WK-HT. Therefore mouse studies showed: (i) a control of leaning frequency in D2R(-/-) and D3R(+/-) in an opposite manner,(ii) an influence of D2R on frequency of leanings prevailing in D2D3(-/-) and of D3R(+/-) prevailing in D2D3R(+/-). Rat studies showed higher frequency of rearings and leanings in hyperactive SHR and WK-HA of shorter leanings in NHE and of longer leanings in WK-HA. In conclusion, this series of studies on rearing and leaning in genetic model systems revealed dissociable traits, differently controlled by DA receptors. This in turn, may help understanding the neural mechanisms of cognitive e non cognitive aspects of behaviour.

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Diet supplementation with donkey milk up-regulates liver mitochondrial uncoupling, reduces energy efficiency and improves anti-oxidant and anti-inflammatory defences in rats

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Dietary PUFA, mainly those of the n-3 family, are known to play essential roles in the maintenance of energy balance and in the reduction of body fat deposition through the up-regulation of mitochondrial uncoupling which is the main source of Reactive Oxygen Species (ROS). We hypothesized that rat supplementation with raw donkey's milk (DM), characterized by low-fat content and higher n3:n6 ratio, may affect energy balance, lipid metabolism, anti-inflammatory and antioxidant/detoxifying defences as compared to animals treated with an iso-energetic amount of raw cow's milk (CM).
Methods: Rats were divided into three groups and received the following treatments for 4 weeks: the first two groups, were allowed to drink raw CM or DM (24 or 47 mL/day, respectively) for 4 weeks, the third group without milk supplement was used as control. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ), energy expenditure, energy balance, body composition, serum triglycerides, cholesterol, lipopolysaccharide (LPS) and TNF-α levels were measured. Hepatic inflammatory and antioxidant/detoxifying parameters, mitochondrial fatty acid oxidation, energy efficiency and oxidative stress were also measured.
Results: CM and DM treatments resulted in similar increase of metabolisable energy, nevertheless DM had no effect on body weight gain but improved animal energy expenditure and decreased energy efficiency, as compared to CM-treated or to control rats. Moreover the increased production of CO_2 and higher O_2 consumption in DM-treated animals associated with a decrease in RQ demonstrated that DM intake improved the ability to utilise fat as metabolic fuel and suggested that, in these animals, almost all of the extra energy was dissipated through an increased metabolic activity. Significant reduction of serum triglycerids (TG) in DM-treated rats (compared to controls), associated to decreased body weight, lipid gain and liver lipids as compared to CM-treated animals.
The hypolipidemic effect produced by DM paralleled with the enhanced mitochondrial activity/proton leakage and with the increased activity or expression of mitochondrial markers namely, Carnitine palmitoyl transferase (CPT) and uncoupling protein 2 (UCP2). The association of decreased energy efficiency with reduced pro-inflammatory signs (TNF-α and LPS levels) with the significant increase antioxidant (total thiols) and detoxifying enzyme activities (glutathione-S-transferase, GST; NADH quinone oxidoreductase, NQO1) in DM-treated animals, indicated that beneficial effects were attributable, at least in part, to the activation of Nrf2 (nuclear factor-2 erythroid related factor 2) pathway.
Conclusions: Presented results demonstrate, for the first time, that dietary supplementation with DM milk increases energy expenditure and decreases body lipid accumulation via the mild augmentation of mitochondrial uncoupling pathway which associated to chemo-protective and anti-inflammatory effects in rodents. However, although the observed biochemical mechanisms produced by DM intake are in good accordance with the reported beneficial effects associated to the intake of foods with higher n3/n6 ratio, nevertheless studies aimed at better understanding of n3 and

n6 PUFA role, as well as peroxisome proliferator-activated receptor (PPARα) involvement, in the beneficial effects produced by DM consumption are in progress.

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Cardioprotection by postconditioning in experimental models of cardiac hypertrophy: spontaneously hypertensive and nandrolone-abuse rats

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Background: Postconditioning (PostC) reduces ischemia/reperfusion (I/R) injury. However in the presence of comorbidities the effectiveness of PostC is reduced. We studied whether PostC can reduce I/R injury in the presence of ventricular hypertrophy induced by hypertension or nandrolone (ND), an anabolic-androgenic steroid. **Methods**: A. Hypertension model: hearts isolated from spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were subjected to: 30-min ischemia and 120-min reperfusion (I/R); I/R+PostC (5 cycles 10s I/R); 4-weeks pretreatment with an angiotensin-converting enzyme inhibitor (ACE-I) before to subject the hearts to I/R with or without PostC maneuvers; ACE-I infusion during early reperfusion (20 or 40 min) with or without PostC maneuvers. B. Drug-abuse: hearts isolated from ND pretreated (14-days or 10-weeks) rats and untreated rats underwent to I/R and PostC protocols. Infarct size and left ventricular pressure were evaluated. WB studies were also performed. **Results and Conclusions**: Data confirm PostC effectiveness in normotensive WKY and PostC ineffectiveness in SHR in limiting infarct size. Chronic ACE inhibition favors infarct size reduction in SHR; yet in WKY ACE-I reduces infarct size, but attenuates infarct-size limiting effects of PostC. ACE-I given in reperfusion does not reduce infarct size and does not recover PostC protection in SHR. In ND groups data show that 14-days ND does not induce hypertrophy and improves the post-ischemic cardiac function via β2-adrenoreceptor involvement with and without PostC. However, 10-weeks ND treatment induces cardiac hypertrophy, increases myocardial susceptibility to I/R injury and abolishes cardioprotection by PostC. ND alters the responses of survival kinases to PostC.

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Relationship between temperature and kinetic properties in rabbit intestinal oligopeptide cotransporter PepT1

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The idea for this study came from the observation that, when studied at room temperature, PepT1 transport currents are similar in two isoforms of fish: seabass and zebrafish (poikilotherms) but much slower in the rabbit (homeotherm). This difference in behavior has led us to hypothesize that the native temperature of each species could condition the kinetic properties of the transporter. Thus the slower kinetics of rabbit PepT1 at 22-24°C could be due to the fact that its physiological working temperature is much higher (around 38°C), differently from that of fish which work at lower temperatures.
Therefore the effects of temperature on the functional properties of the intestinal oligopeptide transporter PepT1 from rabbit have been investigated using electrophysiological methods. The dipeptide Gly- Gln at pH 6.5 was used as substrate. Raising the temperature in the range 20 to 30°C causes an increase in the maximal transport-associated current (Imax) with a Q_{10} close to 4. This is expected because transport mechanism occurs as a cycle of serial steps and, according to Arrhenius theory, higher temperatures will speed up all reactions. Furthermore, higher temperatures accelerate the rate of decline of the presteady-state currents observed in the absence of organic substrate. The voltage dependence of the intramembrane charge movement and of the time constant of decline are both shifted towards more negative potentials by higher temperatures. The shift is due to a stronger action of temperature on the outward rate of charge movement compared to the inward rate, indicating a lower activation energy for the latter process.
Consistently, the activation energy for the complete cycle is similar to that of the inward rate of charge movement.
The electrophysiological properties of the rabbit PepT1, show that they become qualitatively and quantitatively similar to their fish counterpart when both transporters are examined at their physiological body temperature, confirming that the discrepancy in kinetic parameters highlighted in fish and rabbit are adaptative changes in the different species.
Temperature also affects the binding rate of the substrate: the $K_{0.5}$ – V curve is shifted to more negative potentials by higher temperatures, resulting in a lower apparent affinity in the physiological range of potentials. This may be due to the fact that temperature may change the balance between flexibility and structural stability of the protein, affecting the interaction of the substrate with the binding site. A lower affinity at higher temperature is also expected as a consequence of the shorter life time of the transporter state to which substrate binding can occur.
However, the overall efficiency of transport, estimated as the $Imax/K_{0.5}$ ratio is significantly increased at body temperature.