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Programme & Abstracts

Portonovo, Ancona, Italy
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Programme

Wednesday, 18 September

10:00 **Registration** **[Hotel La Fonte]**

14:00 **Pre-meeting Symposium**
[Auditorium Selinunte, Hotel La Fonte]

The Senescent Synapse

Fiorenzo Conti (Ancona)
Introduction

Javier DeFelipe (Madrid, Spain)
The pyramidal neuron in cognition: aging and Alzheimer's disease

Alexej Verkhratsky (Manchester, UK)
Astroglia function in ageing and Alzheimer's disease

Thomas C. Foster (Gainesville, FL, USA)
Altered synaptic plasticity during aging: Role of redox state in Ca^{2+} dysregulation

Gemma Casadesus (Cleveland, OH, USA)
Targeting age-related metabolic dysregulation to prevent AD

18:30 **Opening Cerimony** **[Auditorium Selinunte, Hotel La Fonte]**
Carlo Reggiani (President, SIF)
Fiorenzo Conti (President of the Organising Committee)

Plenary Lecture **[Auditorium Selinunte, Hotel La Fonte]**
Dario DiFrancesco (Milan)
How to make a heart beat: generation and control of cardiac rhythm

19:30 ***Welcome cocktail*** **[Hotel La Fonte]**

Thursday, 19 September

8:00 **Registration** **[Hotel La Fonte]**

8:30-10:30 **Parallel Oral Communications**

Neurobiology and Neurophysiology
[Auditorium Selinunte, Hotel La Fonte]

Chairs: Leonardo Chelazzi (Verona), Luciano Domenici (Pisa)

Roberto Maggi (Milan)
Development of neuroendocrine lineages by differentiation of a stable cell line of mouse fetal hypothalamic neural stem cells (AC1)

Gian Carlo Demontis (Pisa)
Functional characterization of immature precursors of mouse rod photoreceptors

Franca Codazzi (Milan)
Mechanisms of iron uptake in astrocytes under physiological and pathological conditions

Cristina Limatola (Rome)
KCa3.1 channels are involved in the infiltrative behavior of glioblastoma *in vivo*

Rossella Breveglieri (Bologna)
Cells modulated by reaching preparation in area V6A of the monkey posterior parietal cortex

Monica Maranesi (Parma)
Space-dependent representation of objects and other's actions in monkey ventral premotor grasping neurons

Metabolism, Nutrition and System Physiology

[Sala dei Marescialli, Fortino Napoleonico]

Chairs: Giorgio Fano'-Illic (Chieti), Rosa Serio (Palermo)

Fabio Francini (Florence)
Effects of obestatin on smooth muscle from different gastric regions of the mouse

Elena Grossini (Novara)
In anesthetized pigs human chorionic gonadotropin increases myocardial perfusion and function through a β-adrenergic related pathway and nitric oxide

Livio Luzi (Milan)
Role of L-carnitine on skeletal muscle differentiation induction and progression

Andrea Moriondo (Varese)
Role of diaphragmatic skeletal muscles in local lymphatic flux

Enzo Spisni (Bologna)
Dietary Geraniol modulates systemic inflammation and dysbiosis in dextran sodium sulphate-treated mice

Ileana Terruzzi (Milan)
DNA demethylation enhances myoblasts hypertrophy during the late phase of myogenesis activating the IGF-I pathway

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology

[Sala Chiesetta, Hotel La Fonte]

8:30-8:45 **Welcome**

Carlo Reggiani (President, SIF) and Elena Fabbri (President, ESCBPnew)

Session 1

Chairs: Helmut Segner (Bern, Switzerland), Elena Fabbri (Bologna)

8:45-9:25 **Lecture 1**

Luana Ricci Paulesu (Siena)
In vitro effects of environmental endocrine active substances in human placenta

9:30-10:30 **Oral Communications**

Maria Marino (Rome)
Anti-androgenic effects of environmental endocrine disruptors hinge on androgen receptor splice variants

Elena Grasselli (Genoa)
Thyromimetic effects of Tetrabromobisphenol A (TBBPA) on lipid metabolism in steatotic FaO rat hepatoma cells

Anna Palumbo (Naples)
Nitric oxide as mediator of stress response in marine organisms

Silvia Franzellitti (Ravenna)
Serotoninergic signaling in Mytilus galloprovincialis haemocytes and its modulation by fluoxetine

10:30-11:00 ***Coffee break*** **[Hotel La Fonte and Fortino Napoleonico]**

11:00-13:00 Parallel Symposia

Probing neuronal circuitry with non-invasive brain stimulation: new prospects for neurophysiological research

[Auditorium Selinunte, Hotel La Fonte]

Chair: Claudio Grassi (Rome)

Maria Vittoria Podda (Rome)
Modulation of hippocampal plasticity by direct current stimulation in mice: insights from *in vitro* and *in vivo* studies

Carlo Miniussi (Brescia)
Combining electroencephalography and non-invasive brain stimulation offers new prospects in neuroscience

Franca Deriu (Sassari)
Exploring physiological properties of the human facial motor cortex through non-invasive brain stimulation techniques

John Rothwell (London, UK)
New approaches to analyse the behavioural relevance of specific neural circuits in human motor cortex

Protein intake and metabolism in human aging

[Sala dei Marescialli, Fortino Napoleonico]

Chair: Antonio Colantuoni (Naples)

Alberto Battezzati (Milan)
Protein metabolism in aging

Jose Lara (Newcastle, UK)
Age-related changes in weight and body composition: implications for health and aging

Luca Scalfi (Naples)
Evaluation of handgrip strength in human aging

Antonio Colantuoni (Naples)
Protein intake in human aging

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology

[Sala Chiesetta, Hotel La Fonte]

Session 2

Chairs: John Craft (Glasgow, UK), Marcella Motta (Milan)

11:00-11:40 Lecture 2

John Craft (Glasgow, UK)
Comparing the regulation of gametogenesis and spawning in bivalve molluscs

11:45-13:00 Oral Communications

Helmut Segner (Bern, Switzerland)
The role of estrogens in fish immunity

Laura Canesi (Genoa)
Physiological mechanisms of innate immunity in bivalve molluscs: a lesson from environmental stressors

Aldo Viarengo (Alessandria)
Novel mussel haemolymph biomarkers: a system biology approach

Maria Giulia Lionetto (Lecce)
Changes in granulocyte cell volume as novel general biomarker of pollutant exposure in invertebrates

13:00-14:00 ***Lunch*** **[Hotel La Fonte]**

14:00-15:30 **Poster Session 1** **[Hotel Fortino Napoleonico]**
Topic 1: Neurobiology and Neurophysiology
Topic 2: Metabolism, Nutrition and System Physiology
Topic 3: Comparative and Environmental Physiology, Joint Workshop

15:30-17:30 **Parallel Symposia**

From molecules to movement: the impact of key muscle proteins on muscle function and dysfunction
[Auditorium Selinunte, Hotel La Fonte]

Chair: Francesca Grassi (Rome)

Roberto Bottinelli (Pavia)
The cellular and molecular mechanisms underlying skeletal muscle adaptations

Francesca Grassi (Rome)
Acetylcholine receptors and Ca^{2+} signals in maturing myotubes

Marco Linari (Florence)
Motor and cytoskeleton proteins of the sarcomere and associated myopathies

Corrado Poggesi (Florence)
Myopathies and cardiomyopathies associated to sarcomeric protein mutations

Membrane microdomains and physiological modulation of cellular excitability
[Sala dei Marescialli, Fortino Napoleonico]

Chair: Andrea Barbuti (Milan)

Ilaria Rivolta (Milan)
Caveolin-1 expression in lung cells: early sensor of transduction signalling

Andrea Barbuti (Milan)
An altered interaction of caveolin-3 with cardiac ion channels affects cell excitability

Cinzia Volonté (Rome)
Modulation of purinergic signalling at the plasma membrane: a multipurpose and multidirectional way to orchestrate physiopathological conditions in the nervous system

Elisabetta Gazzerro (Genoa)
Caveolinopathies: translational implications of caveolin-3 in skeletal muscle disorders

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology
[Sala Chiesetta, Hotel La Fonte]

Session 3

Chairs: Roy Weber (Aahrus, Denmark), Luana Ricci Paulesu (Siena)

15:30-16:10 **Lecture 3**

Roy Weber (Aarhus, Denmark)
Hemoglobins: models of physiological adaptation, with special reference to O_2 availability and temperature

16:15-17:30 **Oral Communications**

Mariano Beltramini (Padua)
Entrapment of hemocyanin conformers as a tool for the definition of the structural model of cooperativity

Claudio Agnisola (Naples)
Environmental stress and nitrogen excretion in freshwater teleosts

Vittore Verratti (Chieti-Pescara)
The physiological urination mechanism at hypoxic altitudes

Filippo Garofalo (Arcavacata di Rende)
Environmental challenges and organ morpho-functional remodeling: the African lungfish as a case study

Michele Maffia (Lecce)
A protein cold adaptation strategy via a unique seven amino acid domain in the icefish (*Chionodraco hamatus*) PEPT1 transporter

17:30-18:00 ***Coffee break*** **[Hotel La Fonte and Fortino Napoleonico]**

18:00-19:00 **Prizes**
[Auditorium Selinunte, Hotel La Fonte]

SIF Prize

Daniela Puzzo (Catania)
Hippocampal synaptic plasticity and memory: physiological role of amyloid-beta peptide and its implication in Alzheimer's Disease

Farmigea Prize

Paolo Medini (Genoa)
Microcircuits underlying multisensory interactions in the cerebral cortex: primary visual cortex and beyond

Friday, 20 September

8:30-10:30 **Parallel Oral Communications**

Cell Physiology
[Auditorium Selinunte, Hotel La Fonte]

Chairs: Fernando Goglia (Benevento), Maria Svelto (Bari)

Giulia Campostrini (Milan)
Isolating sinoatrial precursors from embryonic stem cells for the development of a biological pacemaker

Massimo Dal Monte (Pisa)
Functional involvement of β3-adrenergic receptors and nitric oxide in melanoma growth

Manuela De Bellis (Bari)
A novel human Aquaporin-4 splice variant exhibits a dominant-negative activity: a new mechanism to regulate water permeability

Antonella Naldini (Siena)
Adaptative cellular responses to hypoxia: role of HIF-1 dependent genes in endothelial cell autophagy and survival

Pasquale Pagliaro (Turin)
Post-conditioning with catestatin (CST-Post) protects the heart of spontaneously hypertensive rats (SHR) from ischemia/reperfusion injury and triggers anti-apoptotic and pro-angiogenetic factors

Feliciano Protasi (Chieti)
Calsequestrin-1 in skeletal muscle: what we learned from knockout animals

Physiology of Motor Systems and Exercise
[Sala dei Marescialli, Fortino Napoleonico]

Chairs: Carlo Reggiani (Padua), Arsenio Veicsteinas (Milan)

Bert Blaauw (Padua)
The role of S6 kinase in the regulation of skeletal muscle mass and function

Paolo Bruseghini (Verona)
Effect of high-intensity-interval-training (HIT) on maximal aerobic power and ventilatory threshold in older adults

Paolo Cavallari (Milan)
The ischemic block of the forearm abolishes index-finger's movements but not its associated APAs

Silvia Spadacenta (Rome)
Arm reaching movements modulate action-related verbs free recall: an embodied memory account

Bruno Grassi (Udine)
O_2 cost of cycling, respiratory muscles training and exercise tolerance in obese adolescents

Vincenzo Lombardi (Florence)
Toward the realization of a sarcomere like machine: force spectroscopy of an ensemble of motors (HMM) from myosin II of frog skeletal muscle

10:30-11:00 ***Coffee break*** **[Hotel La Fonte and Fortino Napoleonico]**

11:00-13:00 Parallel Symposia

Neural plasticity in health and disease: insights from animal models
[Auditorium Selinunte, Hotel La Fonte]

Chair: Nicola Origlia (Pisa)

Maria Spolidoro (Paris, France)
Cerebellar integration in cortical sensorimotor circuits as a substrate for motor coordination plasticity

Nicola Origlia (Pisa)
Entorhinal cortex as a model to study synaptic plasticity in neurodegeneration

Roberto Piacentini (Rome)
Determinants of impaired hippocampal plasticity in experimental models of neurodegenerative diseases

Alessandro Sale (Pisa)
Environmental therapy for plasticity enhancement in a Down Syndrome model

Update in respiratory translational medicine: from alveoli to the whole lung and from experimental model to humans
[Sala dei Marescialli, Fortino Napoleonico]

Chair: Giuseppe Miserocchi (Milan)

Enrico Mazzuca (Milan)
Interaction between alveolar elastic and surface forces studied by *in vivo* microscopy

Daniela Negrini (Varese)
Lung tissue matrix remodeling: correlation with pathophysiology of lung edema in normal and mechanically ventilated lung

Alice Panariti (Milan)
Lung tissue matrix remodeling: mechanisms of lung cellular response to the need for interstitial matrix remodeling

Caterina Salito (Milan)
Functional imaging for the assessment of regional ventilation in health and emphysema

Giuseppe Miserocchi (Milan) and Alessandro Brunelli (Ancona)
Effects of hydrothorax and lobar resection on lung mechanics

13:00-14:00 ***Lunch*** **[Hotel La Fonte]**

14:00-15:30 **Poster Session 2** **[Hotel Fortino Napoleonico]**
Topic 4: Cell Physiology
Topic 5: Physiology of Motor Systems and Exercise

15:30-16:30 **Fabio Ruzzier Lecture** **[Auditorium Selinunte, Hotel La Fonte]**
Hans Hoppeler (Bern, Switzerland)
Malleability of skeletal muscle: performance, structure and mechanisms

16:30-17:00 ***Coffee break*** **[Hotel La Fonte and Fortino Napoleonico]**

17:00 **SIF General Assembly** **[Auditorium Selinunte, Hotel La Fonte]**
Election of the next SIF President

21:00 ***Social dinner*** • edi·ermes
[Hotel Fortino Napoleonico]

Posters

P1.1 *Anna Binda (Monza)*
Study of the effects of nanoliposomes engineered for the treatment of Alzheimer's disease on the electrical activity of cortical neurons

P1.2 *Stefania Bruni (Parma)*
Coding of goal-directed actions in ventrolateral prefrontal and ventral premotor cortex

P1.3 *Simona Capsoni (Pisa)*
Beyond the cholinergic activity of NGF: rescue of Alzheimer-like neurodegeneration by painless NGF acting on APP processing and glial cells

P1.4 *Luca Carnevali (Parma)*
Low vagally-mediated heart rate variability and increased susceptibility to ventricular arrhythmias in rats bred for high anxiety

P1.5 *Elenia Cinelli (Florence)*
Lisinopril, but not losartan microinjected into the caudal nucleus tractus solitarii potentiates the cough reflex in the rabbit

P1.6 *Antonio Colantuoni (Naples)*
Vaccinium Myrtillus anthocyanosides long-term intake and hamster pial microcirculation during ischemia-reperfusion injury

P1.7 *Marianna Crispino (Naples)*
Synaptosomal protein synthesis from rat brain: more than one system?

P1.8 *Giulia Curia (Modena)*
Resilience to audiogenic seizures is associated with p-ERK1/2 dephosphorylation in the subiculum of a mice model of Fragile X syndrome

P1.9 *Maria Egle De Stefano (Rome)*
Early axonal growth of hippocampal neurons is reduced and less sensitive to BDNF in dystrophic mdx mice compared to wild-type

P1.10 *Simone Ferrari-Toniolo (Rome)*
Neural activity associated to joint-action during social cooperation in frontal and parietal cortex of macaque monkeys

P1.11 *Stefano Frausin (Trieste)*
Neural stem cells-enriched tubulization improves anatomical and functional restoration of the severed rat sciatic nerve

P1.12 *Luca Guglielmi (Perugia)*
On the role of K+ channels in autism spectrum disorders

P1.13 *Andrea Mazzatenta (Pisa)*
The human olfactory threshold: new physiological insight on its senescence

P1.14 *Andrea Minelli (Urbino)*
Alpha-tocopherol reduces neuroinflammation in the rat brain after kainic acid-induced status epilepticus

P1.15 *Odysseas Papazachariadis (Rome)*
Local field potentials are influenced by cooperative joint-action in frontal and parietal cortex of macaque monkeys

P1.16 *Rosalba Parenti (Catania)*
Expression of Wilms' Tumor protein (WT1) in developing human peripheral sympathetic and gastroenteric nervous system

P1.17 *Irene Persiconi (Rome)*
Post-natal developmental alterations in the retina of dystrophic mdx mice

P1.18 Onofrio Petruzzelli (BARI)
Identification of anti-neuronal antibodies in the serum of patients with Tourette's syndrome

P1.19 *Francesco Pisani (Bari)*
Identification of a point mutation impairing the binding between Aquaporin-4 and the Neuromyelitis Optica autoantibodies

P1.20 *Daniela Puzzo (Catania)*
F3/contactin promotes hippocampal neurogenesis, synaptic plasticity and memory in adult mice

P1.21 *Margherita Riggi (Trieste)*
Characterization of cognitive deficits in rats with selective cholinergic, noradrenergic and dopaminergic lesions

P1.22 *Alessandro Romano (Lecce)*
Novel domain architecture for sacsin protein using comparative analysis and functional mapping of human SACS mutations

P1.23 *Eleonora Satta (Rome)*
Development of motor cooperation through joint-action

P1.24 *Andrea Sgoifo (Parma)*
Vagal withdrawal and cardiac arrhythmia vulnerability in rats with high trait aggressiveness

P1.25 *Andrea Valeri (Trieste)*
Multiple effects of selective cholinergic lesions combined with local infusion of pre-aggregated amyloid peptide

P1.26 *Tiziano Verri (Lecce)*
Anti-aggregating effect of the naturally occurring dipeptide carnosine on Aβ1-42 fibril formation

P1.27 *Federica Visco-Comandini (Rome)*
A visuomotor disorder in absence of movement: optic ataxia generalizes to learned isometric hand action

P1.28 *Serena Viventi (Trieste)*
Progressive motoneuronal degeneration and motor dysfunction in sod1g93a mice: effects of implanted mesenchymal stem cells from human umbilical cord (HUMSCS)

P2.1 *Alberto Battezzati (Milan)*
Plasma Bisphenol-A concentration and body fat distribution: a pilot study in an urban area

P2.2 *Simona Bertoli (Milan)*
Ketogenic diet: a model to evaluate the effect of high fat diet on regional adiposity and glucose metabolism in humans

P2.3 *Massimo Bramucci (Camerino)*
Antiproliferative and anti-inflammatory activities of the essential oil from fruits of Xylopia parviflora (A. Rich.) Benth. (Annonaceae) used in Cameroon as a culinary spice

P2.4 *Gabriella Gallo (Genoa)*
3,5-L-diiodothyronine (T2) modifies the fatty acid composition of lipid droplets in an in vitro model of hepatosteatosis

P2.5 *Maria Elena Giordano (Lecce)*
Antioxidant effect of a purified polyphenolic extract from grape skin on rat colon epithelium

P2.6 *Fernando Goglia (Benevento)*
Metabolic adaptation induced by triiodotironine: a role for Uncoupling protein-3 (UCP3)

P2.7 *Lilla' Lionetti (Naples)*
Physiological impact associated with chronic simultaneous exposure to high-fat diet and persistent organic pollutant p,p´-diphenyldichloroethene (DDE): effects on hepatic mitochondrial functions

P2.8 *Herbert Ryan Marini (Messina)*
Effects of genistein, a soy-derived isoflavone, on endothelial function in postmenopausal women with metabolic syndrome

P2.9 *Lucia Martinoli (Rome)*
Physical, chemical and morphological changes of bread polyphenol extract on cellular cultures monolayer CaCo-2

P2.10 *Maria Mollica (Naples)*
Metabolic responses to isoenergetic intake of cow, donkey or human milk in rats

P2.11 *Marina Montagnani Marelli (Milan)*
Role of ERbeta in human melanoma cells and its involvement in tocotrienols activity

P2.12 *Espedita Muscariello (Naples)*
Evaluation of cut-off scores for sarcopenia in a clinical obese population

P2.13 *Graziella Santoro (Varese)*
Selective modulation of intrinsic myogenic activity of peripheral diaphragmatic lymphatics by epinephrine

P2.14 *Rosa Serio (Palermo)*
Postnatal development of the 5-hydroxytryptamine (5-HT) signaling system in the mouse duodenum

P2.15 *Emanuela Viggiano (Naples)*
Atypical antipsychotic increases phospho-AMP-activated protein kinase in the hyphotalamus

P3.1 *Maria Cerra (Arcavacata di Rende, CS)*
AngII-dependent modulation of eel heart morpho-functional remodelling

P3.2 *Caterina Ciacci (Urbino)*
Effects of different environmental Vibrio strains on functional parameters of mussel hemocytes

P3.3 *Elena Fabbri (Bologna)*
Adrenergic receptors and signaling in yellow and silver European eel hepatocytes

P3.4 *Alfonsina Gattuso (Arcavacata di Rende, CS)*
Mechanims of hydrogen sulphide signalling in frog and rat hearts: Akt/eNOS phosphorilation and PLN S-Sulfhydration

P3.5 *Laura Grumetti (Naples)*
Nitrite effects on swimming performance in the convict cichlid, Amatitlania nigrofasciata

P3.6 *Paola Irato (Padua)*
Physiological responses to Cd exposure in Mytilus sp.

P3.7 *Alessandro Rubini (Padua)*
Effects of Hyperbaric Oxygen exposure (HBO) in patients before surgical pancreatoduodenectomy

P3.8 *Gianfranco Santovito (Padua)*
Peroxiredoxin 6 and antioxidant defences in Antarctic fish

P3.9 F*rancesca Trischitta (Messina)*
Effect of Sodium Dodecyl Sulfate on RVD of digestive cells of Mytilus galloprovincialis

P3.10 *Paola Valbonesi (Ravenna)*
Acetylcholinesterase activity and expression in PC12 cells exposed to high-frequency electromagnetic fields (GSM 1.8 GHz)

P4.1 *Filippo Acconcia (Rome)*
The physiological role of the estrogen receptor alpha ubiquitin binding surface in 17beta-estradiol-dependent cell proliferation and cholesterol homeostasis

P4.2 *Tommaso Angelone (Arcavacata di Rende, CS)*
Serpinin as a novel CgA-derived cardioprotective peptide

P4.3 *Amilcare Barca (Lecce)*
Dynamic interplay between genes involved in carnosine and copper metabolism under carnosine exposure in neuronal and astrocytic mammalian cells

P4.4 *Pasquale Bianco (Sesto Fiorentino, FI)*
Transient kinetics measured with force steps discriminate between double stranded DNA elongation and melting and define the reaction energetics

P4.5 *Leonardo Bocchi (Parma)*
Cardiac effects of acute exposure to titanium dioxide nanoparticles: electrophysiological characterization

P4.6 *Gerardo Bosco (Padua)*
Effect of hyperbaric oxygen treatment (HBO) and gemcitabine on apoptosis in pancreatic ductal adenocarcinoma cells

P4.7 *Luigi Bubacco (Padua)*
N-ethylmaleimide sensitive fusion protein (NSF) complex formation and its biological regulation: implication for synaptic vesicle exocytosis

P4.8 *Francesca Cacciani (Parma)*
Spontaneous beating of guinea pig sinoatrial cells under pharmacolgical modulation of two different pacemaker mechanisms

P4.9 *Elena Candelotti (Rome)*
Cross-talk between thyroid hormone and IGF-1 in THP-1 monocytes is mediated by integrin αvβ3

P4.10 *Michela Castagna (Milan)*
Threonine 67 as a molecular hinge for the coupling mechanism in the NSS amino acid transporter KAAT1

P4.11 *Simona Damiano (Naples)*
Epidermal growth factor induces MUC3A and MUC5AC mucin expression via dual oxidase-2 dependent reactive oxygen species production in human enterocyte-like cells

P4.12 *Stefania Fulle (Chieti)*
Does apoptosis affect the muscle regeneration in human aged satellite cells?

P4.13 *Adriana Graziano (Catania)*
Role of connexin 43 in the apoptosis induced by psychosine in mouse oligodendrocyte precursors

P4.14 *Vincenzo Lionetti (Pisa)*
Prosurvival communication between endothelial cells and mesenchymal stem cells through exosomes containing HIF1-alpha

P4.15 *Debora Lo Furno (Catania)*
Influence of conditioned medium from neuroblastoma B104 or olfactory ensheathing cells in the differentiation of human adipose stem cells into neural phenotype

P4.16 *Maria Mariggiò (Chieti)*
Role of GAP-43 in myogenesis: the model of myogenic satellite cells from GAP-43 knockout mice

P4.17 *Maria Grazia Mola (Bari)*
Contribution of aquaporins and TRPV4 to astrocyte cell volume regulation

P4.18 *Maria Teresa Nuzzo (Rome)*
Estrogen receptor subtypes are differently requested for E2-neuroprotective effects

P4.19 *Valentina Pallottini (Rome)*
Deregulation of cholesterol biosynthetic pathway in Rett syndrome

P4.20 *Maria Pascale (Fisciano, SA)*
Response of cardiac muscle cells to stressful stimuli: BAG3 protein modulation

P4.21 *Claudia Penna (Orbassano)*
Cardioprotection by ischemic (I-PostC) and pharmacological postconditioning (P-PostC) involve S-nitrosylation (SNO) of mitochondrial proteins: role of RISK and SAFE pathways

P4.22 *Carla Perego (Milan)*
The glutamate signalling in islet of Langerhans: molecular mechanisms of modulation

P4.23 *Proto Pippia (Sassari)*
MASER-12 suborbital space flight mission: effects of altered gravity on signal transduction in primary human T lymphocytes

P4.24 *Elisa Ren (Trieste)*
Adenosine receptors modulate the autocrine nAChR-driven [Ca2+]i spiking activity of in vitro contracting myotubes

P4.25 *Vittorio Ricci (Pavia)*
Seeing is believing: evidence of a novel cytoplasmic structure containing functional proteasome and inducible by cytokines/trophic factors

P4.26 *Stefania Rosito (Bari)*
Involvement of Aquaporin-4 in modulation of glucose transport and glycogen content in fast-twitch muscles in a Calcium-dependent pathway

P4.27 *Giulio Sancini (Monza)*
Solid Lipid Nanoparticles: a strategy to overcome the blood-brain barrier

P4.28 *Monia Savi (Parma)*
Effects of acute exposure to titanium dioxide (TiO2) nanoparticles on ventricular cardiomyocytes: mechanical and cytotoxic characterization

P4.29 *Trifone Schettino (Lecce)*
Preliminary study of gene expression of TJP1, CLDN2 and MYO9B in patients with Systemic Nickel Allergy Syndrome (SNAS)

P4.30 *Pierangela Totta (Rome)*
A role for the ubiquitin-activating enzyme in 17β-estradiol-induced cell proliferation, migration and cholesterol homeostasis

P4.31 *Emanuela Urso (Lecce)*
Insights into the mechanisms underlying copper absorption in human endothelial cells

P4.32 *Barbara Pavan (Ferrara)*
Epithelial barrier impairment in HRPE cells is prevented by Goji berry extracts: cAMP as a potential trigger molecule

P5.1 *Antonio Cevese (Verona)*
Effects of whole body oscillations on cardiovascular variables and spontaneous variability

P5.2 *Barbara Colombini (Florence)*
Crossbridge properties during fatigue and recovery in mammalian intact skeletal muscle fibres at physiological temperature

P5.3 *Maristella Gussoni (Segrate, MI)*
A quantitative method to monitor short and long-term Reactive Oxygen Species (ROS) production kinetics in humans by Electron Paramagnetic Resonance (EPR)

P5.4 *Fiorenzo Moscatelli (Foggia)*
Excitability of primary motor cortex in karate athletes: a transcranial magnetic stimulation study

P5.5 *Vincenzo Perciavalle (Catania)*
Blood lactate levels and visual evoked potentials

P5.6 *Gabriella Piazzesi (Sesto Fiorentino, FI)*
Temperature dependence of myosin filament structure in muscle and in skinned fibres from mammals

P5.7 *Stefano Sartini (Urbino)*
Motor activity and muscle re-innervation: the influence of different patterns of exercise

P5.8 *Marina Sciancalepore (Trieste)*
“Noisy”-patterned extracellular electrical stimuli facilitate muscle cell contractions in vitro

Abstracts

Oral Presentations
(in chronological order of presentation)

Development of neuroendocrine lineages by differentiation of a stable cell line of mouse fetal hypothalamic neural stem cells (AC1)

A. Cariboni, L. Conti, V. Andrè, D. Aprile, J. Zasso, R. Maggi
Dept Pharmacological and Biomolecular Sciences, CIRMAR, Univ. degli Studi di Milano

The mammalian hypothalamus is involved in regulating several physiological functions exerted by the neuroendocrine system. The neuroendocrine hypothalamus contains two distinct neuronal subsystems characterized by the production of specific hormonal neuropeptides; however, the molecular pathways that mediate the development of such neurons are largely unknown.
The present communication describes the setup and the characterization of a pure stable cell line of neural stem cells from E12 fetal mouse hypothalamus. The cell line (named AC1) grows as a monolayer in continuous expansion, by symmetrical division, in a defined medium enriched in FGF-2 and EGF. They express stemness (nestin, Sox-2 and Pax-6), neuronal (β 3-tubulin), but not astrocytic (GFAP), markers; moreover, AC1 show the expression of some hypothalamic patterning genes (Sim1, Sim2, Arnt2, Brn2 and Mash1). After prolonged expansion they remain able to differentiate efficiently into neurons and astrocytes *in vitro*. In normal culture conditions, AC1 do not express detectable levels of hormonal neuropeptides; however, transcripts for TRH, CRH and POMC were evident after induction of neuronal differentiation with morphogens. The ability of AC1 cells to develop neuroendocrine lineages in vitro will help to elucidate the mechanisms involved in the physiological hypothalamic developmental processes as well as in the specific differentiation of neurohormonal hypothalamic neurons (*granted by MIUR*).

Parallel Oral Communication
Neurobiology and Neurophysiology

Functional characterization of immature precursors of mouse rod photoreceptors

G.C. Demontis[1], S. Giannelli[2], S. Tropea[1], V. Broccoli[2]
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Rod photoreceptors produce and release cone viability factors, and selective rods demise due to genetic defects is followed by cone photoreceptors loss, up to legal blindness. Generation of rod precursors by genetic reprogramming of non-neuronal cells is required for the discovery, by high-throughput screening, of drugs preventing rod demise. However, no data are available on the functional properties of either immature retinal rod precursors or rod precursors derived from non-neuronal cells by genetic reprogramming. We present here the first functional characterization by patch-clamp recordings of immature postmitotic rod precursors, isolated from the retina of Nrl-EGFP transgenic mice. Our results indicate they express most currents typical of adult rods, including a large Cs-sensitive hyperpolarization-activated current (I_h), a transient outward potassium current (I_{Kx}) and a large transient inward current similar to $I_{Cl(Ca)}$. The analysis of current kinetics reveals several differences between adult rods and their immature precursors. These functional data will assist the development of functional rod precursors from reprogrammed cells, enabling the search for drugs able to prevent rods demise and the ensuing loss of high resolution cone-mediated vision.

Mechanisms of iron uptake in astrocytes under physiological and pathological conditions

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Astrocytes play a crucial role in CNS iron homeostasis since they regulate a number of physiological functions but can also be involved in neurodegenerative processes.
Primary rat hippocampal astrocytes were analyzed by a comprehensive panel of experimental approaches in order to characterize iron entry pathways and iron content both at rest and after activation (24 h exposure to IL1−β and TNFα).
We show that the major source of iron in astrocytes is represented by the non−transferrin−bound iron and demonstrate the involvement of two different routes for its entry. The opening of resident transient receptor potential canonical (TRPC) channels accounts for iron uptake in quiescent astrocytes, with a potentially relevance at the synaptic level, where glutamate spill over can favour TRPC activation. On the other hand, astrocyte activation promotes a raise of divalent metal transporter 1 (DMT1) expression with potentiation of iron entry and accumulation. Our results also indicate that the DMT1−1A/IRE(+), highly localized at the plasma membrane level, is the main DMT1 isoform involved in this process.
Overall, our data suggest that at rest, but even more after activation, astrocytes have the competence to buffer the excess of extracellular iron, thereby protecting neurons from iron overload. These findings further extend our understanding of the protective role of astrocytes under the conditions of iron−mediated oxidative stress observed in several neurodegenerative conditions.

Parallel Oral Communication
Neurobiology and Neurophysiology

KCa3.1 channels are involved in the infiltrative behavior of glioblastoma *in vivo*

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Glioblastoma multiforme (GBM) is a diffuse brain tumor characterized by high infiltration in brain parenchyma rendering the tumor difficult to eradicate by neurosurgery. Efforts to identify molecular targets involved in the invasive behavior of GBM suggested ion channel inhibition as a promising therapeutic approach. To determine if the Ca^{2+}-dependent K^+ channel KCa3.1 could represent a key element for GBM brain infiltration, human GL-15 cells were xenografted into the brain of SCID mice which were then treated with the specific KCa3.1 blocker TRAM-34. After five weeks of treatment, we observed a reduced tumor infiltration and astrogliosis around the tumor, compared to untreated mice. Reduction of tumor infiltration was also observed in the brain of mice transplanted with KCa3.1-silenced GL-15 cells, indicating a direct effect of TRAM-34 on GBM-expressed KCa3.1 channels. Since KCa3.1 channels are also expressed on microglia, we investigated the effects of TRAM-34 on microglia activation in GL-15 transplanted mice and found a reduction of CD68 staining in treated mice. Similar results were observed *in vitro* where TRAM-34 reduced both phagocytosis and chemotactic activity of primary microglia exposed to GBM conditioned medium.
These results indicate that KCa3.1 activity plays an important role in GBM invasiveness *in vivo* and that its inhibition directly affects glioma cell migration and reduces astrocytosis and microglia activation in response to tumor-released factors.

Cells modulated by reaching preparation in area V6A of the monkey posterior parietal cortex

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Area V6A is involved in the monitoring of reaching movements. During foveal reaching tasks, many V6A cells show modulations in the delay before reaching that could be related to eye position signals and/or reach planning. Here we want to study the relative contribution of these signals. Single cell activity was recorded in two *Macaca fascicularis* while they executed in darkness a detection task and a reaching task. In the reaching task, body-out arm movements were executed after a delay towards a foveated target, whereas in the detection task the monkeys just fixated the target. We found cells where delay activity was equally spatially tuned in the two tasks and are likely to encode spatial location (Gaze cells 41/162, 25%), cells spatially tuned only during reaching preparation (Set cells 27/162, 17%), and cells differentially spatially tuned in the two tasks (Gaze/Set cells 71/162, 44%). In cells influenced by reaching preparation, the delay activity in the reaching task could be higher or lower compared to the detection task. All Set cells and a minority of Gaze/Set cells were more active during reaching preparation; the majority of Gaze/Set cells discharged less during that period. Present results highlight the convergence of visuospatial and reach planning signals on V6A and suggest that visuospatial processing can have a larger influence on V6A activity than the encoding of reach plans. Grants: FP7-ICT-217077-EYESHOTS, Fondazione del Monte di Bologna e Ravenna, MIUR.

Parallel Oral Communication
Neurobiology and Neurophysiology

Space-dependent representation of objects and other's actions in monkey ventral premotor grasping neurons

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Monkey ventral premotor area F5 contains different classes of grasping neurons. Purely motor neurons discharge only during grasping execution, while visuomotor neurons also show visual responses to the presentation of graspable objects (canonical neurons) or to the observation of other's grasping acts (mirror neurons). Canonical and mirror neurons are deemed to form anatomo-functionally distinct populations, but no study has directly investigated the possible interaction between visual information on objects and observed actions at the single neuron level. To this purpose, we simultaneously recorded neuronal activity by means of 16 channels linear probes in 2 monkeys while they performed a visuo-motor task and observed an experimenter doing the same task. We recorded 464 neurons: 219 were classified as purely motor and 245 as visuomotor. Among these latter, 137 responded during action observation, 46 during object presentation and 62 to both types of stimuli. Interestingly, visual responses to objects were mostly limited to the monkey peripersonal space, while those to other's actions could be present in peri- and/or extrapersonal space. Further experiments revealed that responses to objects rely on a pragmatic rather than metric representation of the peripersonal space. These results suggest the existence of a space-gated mechanism for the representation of objects and actions in the same premotor neurons, enabling the system to save neuronal resources.

Effects of obestatin on smooth muscle from different gastric regions of the mouse

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Aim: Obestatin is a peptide hormone released from the stomach known to be active in decreasing food intake. It has been already observed that it modulates cholinergic neurotransmission at the postganglionic site and acts on gastric fundal smooth muscle. In order to assess if a differential function exists for obestatin in different regions of the stomach, the aim the of this work was to investigate whether this peptide may differently act on the smooth muscle from gastric fundus and antrum.
Methods: Changes in isometric tension of muscle strips were recorded via force displacement transducers. Voltage dependent ionic currents were recorded in current- and voltage-clamp conditions by single microelectrode inserted in a gastric smooth muscle cell.
Results: In both fundal and antral strips, obestatin caused a decrease of the basal tension. Our electrophysiological experiments, showed that obestatin increased the membrane resistance, caused the inhibition of Ca^{2+} currents and positively shifted their voltage threshold of activation on gastric fundus. Although antral smooth cells showed higher resistance and higher cell capacitance, obestatin caused similar effects on antrum.
Conclusion: Our results indicate that obestatin directly influences the gastric smooth muscle. The decay of the basal tension caused by obestatin, due at least in part to the inhibition of Ca^{2+} currents, might contribute undeniably to the distension of the gastric wall, both in antrum and in gastric fundus. This event is commonly supposed to be one of the major signal involved in the regulation of food intake.

Parallel Oral Communication
Metabolism, Nutrition and System Physiology

In anesthetized pigs human chorionic gonadotropin increases myocardial perfusion and function through a β-adrenergic related pathway and nitric oxide

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Although human chorionic gonadotropin (hCG) has been reported to elicit cardiovascular effects, information about any direct action on cardiac function, perfusion and related mechanisms has remained scarce. Therefore, the present study aimed to determine the primary *in vivo* effect of hCG on cardiac contractility and coronary blood flow and the involvement of autonomic nervous system and nitric oxide (NO). Moreover, in coronary endothelial cells (CEC) the intracellular pathways involved in the effects of hCG on NO release were also examined. In 25 anesthetized pigs, intracoronary 500 mU/ml hCG infusion at constant heart rate and aortic blood pressure increased coronary perfusion and function and coronary NO release ($P<0.0001$). Moreover, while blockade of muscarinic cholinoceptors (n=5) and of α-adrenoceptors (n=5) did not abolish the observed responses, β_1-adrenoceptors blocker (n=5) prevented the effects of hCG on cardiac function. In addition, β_2-adrenoceptors (n=5) and NO-synthase inhibition (n=5) abolished the coronary response and the effect of hCG on NO release. In CEC, hCG induced the phosphorylation of endothelial NO synthase through cAMP/PKA signalling and ERK1/2, Akt, p38MAPK involvement, which were activated as downstream effectors of β_2-adrenoceptors stimulation. In conclusion, in anesthetized pigs hCG primarily increased cardiac function and perfusion through the involvement of β-adrenoceptors and NO release. Moreover, cAMP/PKA-dependent kinases phosphorylation was found to play a role in eliciting the observed NO production in CEC.

Role of L-carnitine on skeletal muscle differentiation induction and progression

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L-Carnitine (CARN) is a conditionally essential nutrient and plays an important role in mitochondrial β-oxidation and in the ubiquitin-proteasome system regulation. Muscle cells are unable to synthesize CARN. As a dietary supplement to ameliorate pathological condition characterized by myofibrils degeneration or improve athletic performance, CARN has been studied for its potential to enhance β-oxidation. However, CARN effects on myogenesis remain unknown. Myogenic Regulatory Factors (MRFs) and late myogenic protein Myosin Heavy Chain (MyHC) coordinate skeletal muscle differentiation.
In this work, the role of CARN on skeletal muscle differentiation induction and progression was investigated using an in vitro mouse myoblasts C2C12 cell line. By Western Blot and Immunofluorescence analysis, we analyzed muscle proteosynthesis and morphological features in C2C12 myoblasts exposed to 5 mM CARN using no CARN stimulated cells as control.
During proliferation phase, CARN positive regulated ameliorated the kinetics of C2C12 cell growth curve and increased early MRFs (MyoD) protein content.
During differentiation, CARN treatment increased the content of MyHC and induced morphological changes indicating the start of hypertrophy process.
These data provide an additional confirm of the potential therapeutic use of CARN to treat pathological condition associated to muscle damage. In particular improving myogenesis, CARN can act in muscle regeneration phenomena.

Parallel Oral Communication
Metabolism, Nutrition and System Physiology

Role of diaphragmatic skeletal muscles in local lymphatic flux

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The passive change in diameter of linear diaphragmatic lymphatics during contraction of adjacent skeletal muscle fibers (extrinsic mechanism) was studied *in vivo* in anaesthetized, paralyzed and ventilated Wistar rats. Maximal contractions of diaphragmatic muscle fibers was induced by local injections of 9.6 nl of KCl 1M solution in proximity to the observed lymphatic vessels which were video recorded for subsequent analysis. In lymphatic vessels running perpendicular to muscle fibers, contraction caused a decrease in diameter to 66.2 ± 1.7 % of resting value in vessels running within a 300mm distance from contraction site, and an increase to 131.1 ± 2.3 % in vessels located at a distance > 900 μm. Vessels parallel to muscle fiber direction do not seem to be involved in lymph propulsion. Both the ejection fraction (54.7 ± 2.3 % vs. 42.9 ± 17 %, $p<0.01$ n=156) and stroke volume (46.2 ± 3.4 pl vs. 34.9 ± 3.0 pl, $p<0.05$, n=156) were larger in passive lymphatics subject to extrinsic skeletal muscle contraction, than in the few peripheral lymphatics able to intrinsically contract. Based on stroke volume and respiratory frequency, the lymph flux sustained by extrinsic mechanism was ~ 2158 ± 158 pl/min, ~ 10 fold higher than that sustained by the intrinsic pumping mechanism (199 ± 14 pl/min). Our data suggest that diaphragmatic lymph flux is heavily dependent on diaphragmatic contractions, leaving a quantitatively less role to the intrinsic pumping mechanism, albeit present.

Dietary Geraniol modulates systemic inflammation and dysbiosis in dextran sodium sulphate-treated mice

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Geraniol is an acyclic monoterpene alcohol with well known anti-inflammatory anti-tumoral, and anti-microbial proprieties. It is widely used as food preservers by food industries and as antimicrobial agents by agricultural industries.
Inflammatory Bowel Diseases (IBDs) are chronic diseases of unknown aetiology that affect the gastro-intestinal tract with an increasing prevalence in western countries. IBDs are characterized by an unbalanced inflammatory response of gut mucosa immune system, associated with alterations in the composition and/or density of the intestinal microbiota, called dysbiosis. Dextran sodium sulphate (DSS)-induced colitis is one of the most commonly used animal model of colitis since it reflects many of the clinical features of IBDs.
The present study investigated the role of Geraniol as anti-inflammatory and anti-dysbiotic agent in vitro and in the model DSS-induced colitis, in mice. Geraniol was orally administrated to C57BL6 mice at the doses 30 and 120 mg $kg^{(-1)}$/body weight, starting six days before DSS treatment and ending the day after DSS removal.

Parallel Oral Communication
Metabolism, Nutrition and System Physiology

DNA demethylation enhances myoblasts hypertrophy during the late phase of myogenesis activating the IGF-I pathway

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Skeletal muscle regeneration and hypertrophy are important responses to both physical activity and pathological stimuli. Recently, research was focalized on understanding epigenetic modifications that coordinate the myogenic lineage acquisition. We have demonstrated that DNA demethylation promotes myogenesis induction. In this study, we explored demethylation effects during myoblasts differentiation and early stage of hypertrophy, using DNA-demethylating agent 5-azacytidine (AZA).
We induced differentiation in C2C12 myoblasts enriching growth or differentiation medium with 5µM AZA. To study hypertrophic process, we stimulated neo formed myotubes with AZA for 24h. Unstimulated cells were used as control. By Western blot and immunofluorescence we examined AZA action on Myogenic Regulatory Factors expression, hypertrophic signaling pathway and myotubes morphology.
During differentiation, protein levels of myogenic markers and Myosin Heavy Chain (MyHC) were higher in AZA stimulated cells compared to control. Immunofluorescence analysis revealed morphological changes in myotubes reflecting a tendency to hypertrophy. In AZA stimulated neo formed myotubes, we observed that IGF-I/p70S6K and ERKs pathways were activated and MyHC protein content was increased.
Our work demonstrates that DNA demethylation could play an important role in promoting the late phase of myogenesis, activating endocellular pathways involved in protein increment and stimulating the hypertrophic process.

In vitro effects of Environmental Endocrine Active substances (EASs) in human Placenta

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Environmental Endocrine Active substances (EASs) are chemicals of agricultural or industrial origin which may influence human reproductive health. The effects of these substances in the prenatal life is an important topic that is receiving greater attention in the developed countries. We focused our attention on the effects of Bisphenol A (BPA) and *para*-nonylphenol (*p*-NP), two EASs with a known estrogenic activity, in human placenta. Primary cultures of chorionic villous explants were used to investigate the effects of chemicals on pivotal physiological processes during placenta establishment and development. BeWo and HTR-8/Sv-neo cells were used to discern effects on trophoblast. Chemicals were used at non-toxic, physiologically relevant, concentrations (10^{-11}M-10^{-9}M). Results showed that both chemicals, were increasing β-hCG and caspase-3, respectively markers of the formation of syncytiotrophoblast and its apoptotic shedding. Both chemicals affected also trophoblast differentiation into the extravillous pathway by reducing cell migration and cell invasion while increasing cell endoreduplication. From the results obtained, we can state that environmental chemicals may interfere with physiological processes in placentation. Even though it is difficult to translate the data obtained in vitro with the pathophysiological conditions, the effects sustained at concentrations as low as nM to pM point to the need for protecting both pre-natal life and development of the foetus.

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology
Oral Communications

Anti-androgenic effects of environmental endocrine disruptors hinge on androgen receptor splice variants

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The increase in non-communicable pathologies in humans and wildlife over the past 40 years indicates an important role of the environment in disease etiology. Endocrine disrupting chemicals (EDCs), a heterogeneous class of man-made and natural compounds, are nowadays recognized as an important component of the environmental influences on disease because they are able to mimic or disrupt the functioning of hormones, principally sex steroid hormones. Until very recently, most of the interest in EDCs was concentrated on their estrogenic activities, although the anti-androgenic properties of some xenobiotics have also been reported. We have used naringenin (Nar) and bisphenol A (BPA) as prototypes of natural and synthetic xenoestrogenic EDCs and evaluated their effects on mouse, rat, and human androgen receptor (AR)-positive cells. Intriguingly, Nar and BPA act as antiandrogen only when AR splicing forms are expressed (i.e prostate cancer cells). These data have been confirmed in HeLa cells transiently transfected with AR wild type (110 kDa) or AR mutants (i.e., AR ~80 kDa and AR ~37 kDa). As a whole, these results support the idea that fewer chemicals possess androgenic activity than they possess estrogenic activity and that interference occurs expecially when AR splicing forms lack the *N*-terminal domain. In addition, estrogen receptors are expressed in male tissues; thus, an overall estrogenic effect would occur in the presence of EDCs and change the male hormonal milieu, which is assured by a characteristic ratio of androgens to estrogens.

Thyromimetic effects of Tetrabromobisphenol A (TBBPA) on lipid metabolism in steatotic FaO rat hepatoma cells

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Tetrabromobisphenol A (2,2-bis(3,5-dibromo-4-hydroxyphenyl) propane, TBBPA) is the most produced brominated flame retardant commonly detected in the environment and in biological samples. TBBPA shares structural similarities with thyroid hormones (THs), and it has been shown to interfere with different aspects of TH signaling, with both thyroidogenic as well as TH antagonist activity, this raising concern on its possible adverse effects as an endocrine disruptor on humans and wildlife. THs play an important role in lipid metabolism, particularly in the liver. In this work, the possible effects of TBBPA in lipid loaded (steatotic) rat hepatoma FaO cells were investigated in comparison with those of T_3 (3,3',5-triiodo-L-thyronine). Lipid accumulation was evaluated in terms of intracellular triglyceride (TAG) content. Transcription of peroxisome proliferator activated receptors (PPAR) isoforms α, γ, and β/δ and of representative PPAR target genes was assessed. TBBPA reduced lipid accumulation in steatotic cells to control levels with effects comparable with those of equimolar doses of T_3. TBBPA and T_3 showed both common and distinct effects on transcription of genes involved in lipid homeostasis. The results demonstrate that TBBPA can decrease lipid accumulation in the hepatic cell mainly through induction of the expression of genes involved in mitochondrial oxidative pathways. These data indicate novel thyromimetic actions of TBBPA.

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology
Oral Communications

Nitric oxide as mediator of stress response in marine organisms

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Nitric oxide is an important physiological messenger present in all metazoans from placozoans to vertebrates. In non-vertebrates nitric oxide is implied in many processes, including development, feeding, defence, bioluminescence, neural transmission. There is also increasing evidence indicating that nitric oxide acts as a cellular signal of environmental stress. Here, we investigated the involvement of this messenger in mediating the cellular response to environmental stress agents, such as metal ions and the diatom-derived toxin decadienal, using different marine organisms. The sea urchin *Paracentrotus lividus* fertilized eggs were treated with cadmium and manganese under different endogenous nitric oxide levels and various developmental stages were examined at morphological, protein and gene levels. Under reduced levels of nitric oxide, an increased number of abnormal plutei was observed suggesting a protective role of this messenger in the stress response induced by these agents. The effect of decadienal was studied on *Ciona intestinalis* development, by following tail regression and juvenile formation. Treatment of hatched larvae with decadienal resulted in a delay of metamorphosis with a concomitant reduction of ERK phosphorylation. The same effect was observed when hatched larvae developed under reduced nitric oxide levels. Overall these studies provide new insight into the role of nitric oxide as a mediator of stress response in marine organisms.

Serotoninergic signaling in *Mytilus galloprovincialis* haemocytes and its modulation by fluoxetine

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Pharmaceuticals are widely detected in aquatic environments, representing a possible threat for aquatic wildlife. Since they are designed to act on specific molecular targets, pharmaceuticals can be effective at low concentrations in aquatic organisms in which the drug targets are evolutionary conserved.
In vivo exposure of Mediterranean mussels to the antidepressant fluoxetine (FX) decreased mRNA levels of the ABCB gene encoding P-glycoptrotein in digestive glands and haemocytes. This membrane transporter is a key component of the mussel cytoprotective system, allowing these animals to cope with environmental stressors. In mammals *ABCB* transcription is under a cAMP/PKA regulation.
The aim of this work was to gain a better knowledge on 5HT transduction pathway in mussels, including its involvement in *ABCB* regulation. Indeed, FX acts as a selective serotonin (5HT) reuptake inhibitor increasing 5HT levels in the synaptic cleft. Haemocytes were treated *in vitro* with 5HT and pharmacological modulators of the cAMP/PKA system. *ABCB* mRNA expression follows induction and inhibition of PKA activity. Indeed, dbcAMP increased PKA activities and *ABCB* mRNA levels; these effects were abolished by the PKA inhibitor H89. *ABCB* was also up-regulated activating the cAMP/PKA signaling through the AC activator forskolin. 5HT reduced cAMP levels and PKA activity, as well as *ABCB* mRNA expression, consistently with the mRNA expression of a $5HT_1$ receptor observed in mussel haemocytes.

Parallel Symposia
Probing neuronal circuitry with non-invasive brain stimulation:
new prospects for neurophysiological research

Modulation of hippocampal plasticity by direct current stimulation in mice: insights from *in vitro* and *in vivo* studies

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Transcranial direct current stimulation (tDCS) is regarded as promising tool to modulate neuronal plasticity for the treatment of neuropsychiatric and neurodegenerative diseases. As such it is mandatory to gain insights on the mechanisms underlying tDCS effects which are largely unknown. Our studies focused on tDCS' effects on long-term potentiation (LTP) at hippocampal CA3-CA1 synapses in the rodent brain. We found that anodal DCS applied *in vitro* to hippocampal brain slices increased LTP while cathodal DCS decreased it. These changes were accompanied with modulation of early gene, zif-268 and c-fos, expression (Ranieri et al., 2012).
Polarity-dependent LTP modulation by tDCS was confirmed in slices obtained from mice stimulated *in vivo*. Furthermore, animals undergoing anodal tDCS showed improved learning and spatial memory. Molecular analysis revealed that anodal tDCS induced histone H3K9 acetylation of specific promoters of BDNF gene. This effect was associated with increased expression of exon I and IX mRNA, whose levels are reportedly elevated during neuronal activation and memory formation. Our novel findings suggest that epigenetic mechanisms modulating BDNF gene expression mediate tDCS-effects on plasticity and memory functions. Our studies shed light on molecular and functional mechanisms potentially dysregulated in a number of brain disorders that can be the target of tDCS-based therapeutic strategies.

Parallel Symposia
Probing neuronal circuitry with non-invasive brain stimulation:
new prospects for neurophysiological research

Combining electroencephalography and non-invasive brain stimulation offers new prospects in neuroscience

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The combination of non-invasive brain stimulation with simultaneous electrophysiological brain imaging, TMS\tDCS-EEG co-registration (repetitive transcranial magnetic stimulation –TMS; transcranial direct current stimulation tDCS), has potential to be of great value for understanding human brain function. It provides real time information about the state of the cortex activity, its functional connectivity, and how brain stimulation modifies such activity and connectivity. Moreover several EEG-TMS studies have explored the possibility of inducing frequency-specific effects by rhythmic TMS to modify behaviour, peripheral responses, or to define the relation between the oscillatory state of the cortex and the response to an upcoming stimulus. Given the recent advances in EEG research, the ability to modulate brain activity is particularly timely and promises to have a huge impact across many domains of clinical and basic neuroscience. In this respect, the possibility to induce neuromodulatory effects by rTMS or tDCS holds considerable promise not only for advancing our understanding of brain rhythms but also in designing new neurorehabilitation strategies. The present talk will consider what and how new information can be gained by integrating these two approaches to investigate the functional state, hierarchy and connectivity of cortical brain areas.

Parallel Symposia
Probing neuronal circuitry with non-invasive brain stimulation:
new prospects for neurophysiological research

Exploring physiological properties of the human facial motor cortex through non-invasive brain stimulation techniques

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Despite the importance of facial muscles in a large spectrum of distinct functions and their frequent and disabling involvement in stroke or movement disorders, conclusive data concerning features of the cortical command to lower facial muscles, role of emotional inputs in facial motor control as well as sensory-motor integration or modulation of plasticity of synaptic connections in the motor cortex innervating facial muscles, are not available yet. Non-invasive transcranial stimulation methods were used to investigate TMS-induced motor evoked potentials, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), short- and long-afferent inhibition after facial nerve electrical stimulation, paired associative stimulation (PAS) and direct current stimulation (tDCS) effects in relaxed and active depressor anguli oris muscles.
Data showed that: i) cortico-bulbar projection to lower facial muscles is bilateral and asymmetric, with contralateral predominance; ii) SICI and ICF operate bilaterally in resting and active states and show a similar extent following stimulation of both hemispheres; iii) afferent inhibition occurs only at long-latency but not at short-latency intervals; iv) a long-term potentiation phenomenon is present after PAS and anodal tDCS, thus proving for the first time that excitability of the facial motor cortex is prone to plastic changes. These findings may provide further physiological insight into pathologies of the facial motor system.

Parallel Symposia
Probing neuronal circuitry with non-invasive brain stimulation:
new prospects for neurophysiological research

New approaches to analyse the behavioural relevance of specific neural circuits in human motor cortex

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Recent work in animals has begun to determine the role of identified types of cortical neuron in particular behavioural tasks (Kvitsiani et al, Nature (2013) 498:363). New developments in non-invasive brain stimulation suggest that a similar approach may become possible even in the human brain. A common protocol to examine neuroplasticity in the motor cortex is paired associative stimulation (PAS) in which repeated pairs of stimuli are applied to median nerve and motor cortex. This leads to long lasting changes in corticospinal excitability involving initial stages of synaptic plasticity at synapses in the cortex. We have recently proposed that the sets of synapses that are strengthened when the interval between median nerve and cortex is 21.5ms is different to the set strengthened at 25ms (Hamada et al, Cerebral Cortex (2013) 23: 1593). In a new set of experiments we show that PAS21.5 and PAS25 interact differentially with two different sorts of motor learning. PAS21.5 (but not PAS25) improves the rate of learning in a ballistic thumb abduction task, whereas PAS25 (but not PAS21.5) reduces the rate of visuomotor adaptation in a visually guided thumb movement task. We conclude that two sets of cortical synapses are involved in each of these particular forms of behavior. The data are relevant to interpretation of pathological changes in response to plasticity protocols in neurological disease.

Parallel Symposia
Protein intake and metabolism in human aging

Protein metabolism in aging

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The human lifespan is progressively increasing in advanced societies but elderly individuals become frail and often disabled by chronic degenerative diseases, causing a significant overlap between what could be defined physiologic and pathologic functioning. A clear focus on physiologic protein metabolism in aging is required in this respect because of the broad clinical implications for sarcopenia, immune dysfunction and fraility. Protein mass is a dynamic pool whose turnover can be quantified, but whose changes in healthy elderly may be too small to detect with current methods and still capable to produce significant losses over years. Evidence of insulin resistance, high splanchnic extraction of dietary amino acids and of the consequent reduction of their incorporation in muscle proteins lead to the concept of "anabolic resistance". In addition, the reduction of anabolic stimuli related to physical activity, dietary intake and to the anabolic hormones environment (Estrogens, Testosterone, Growth Hormone and IGF1) may reduce protein anabolism per se. On the opposite side of protein balance, acute and chronic inflammatory and catabolic responses associated to the frequent comorbidities in the aged may increase protein losses.
Beyond the "classic" determinants of protein metabolism, other nutritional players may exert an impact greater than previously believed on protein metabolism, specifically affecting the regulation of muscle physiology. This is the case of Vitamin D, whose increasingly recognized deficiency affects muscular metabolism and insulin sensitivity beyond the well recognized effects on bone mineralization.
In conclusion, specific differences in protein metabolism of young and older adults mainly refer to anabolic defects of various nature. Changes in endocrine environment, inflammation and nutritional status may produces further changes. New studies support hypotheses relating the losses of muscular and bone tissue in the elderly.

Age-related changes in weight and body composition: implications for health and ageing

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The ageing process is plastic and is driven by the accumulation of molecular damage leading to changes in cell and tissue function caused by chronic exposure to stresses including inflammation, metabolic stress and oxidative stress/ redox changes. A gradual loss of the homeostatic mechanisms necessary to maintain tissue function and physiological capacity is thus a hallmark of ageing.
At the level of body composition, ageing is associated with progressive changes in body composition including fluid re-distribution and reciprocal changes in fat mass and lean body mass. The rate and extent of these changes is influenced by physiological factors such as gender and degree of adiposity of the individual. However it is unclear whether the age-related trajectories of body compartments are determined by genetic and early life exposures or they are continuously subjected to the influence of environmental and physical life-course events. Muscle mass declines 1% per year, however accelerated decline rates may have important clinical consequences and favour the development of sarcopenia and cachexia. There is loss of muscle fibre, particularly fast contracting Type II fibres, decrease in synthesis of contractile protein and reduction of mitochondrial mass.
With an ageing population growing worldwide, it is critical to develop effective interventions to promote healthy ageing and prevent the negative ageing-related body composition changes are critical.

Parallel Symposia
Protein intake and metabolism in human aging

Evaluation of handgrip strength in human aging

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Functional tests are used not only to detect changes due to long-term nutritional deficiencies but also to assess less apparent relationships of nutritional status with health as well as risk factors of chronic diseases. Skeletal muscle force is a physiological function that may be measured in community-based surveys. In such cases, handgrip strength (HGS) is often used as a dynamic indicator of muscle mass.
Actually, HGS largely varies among individuals depending upon a number of factors such as age, gender, and the occurrence of a pathological condition. Reduced HGS is associated with low physical fitness, poor outcome in acute or chronic diseases, and ,especially in the elderly, loss of physical functions and higher morbility and mortality.
Quite surprisingly, not so much attention has been paid to the relationships between HGS and body composition, making more difficult comparing HGS values between individuals and groups. The objective of our study was to explore in elderly individuals the relationships between HGS and field-methods for evaluating body composition. BIA variables such as bioimpedance index, multifrequency ratios and phase angle emerged much stronger than anthropometric measures as significant predictors of HGS in a similar way for the dominant and non dominant sides of the body. These results support the hypothesis that assessing HGS and body composition at the same time may be an useful procedure for nutritional surveillance in elderly people.

Protein intake in human aging

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Protein intake in healthy aging peoples is usually recommended in the range 0,7-0,9 g/Kg/die (1). However, several authors questionned the current Recommended Daily Allowance (RDA) for proteins, because this recommended intake did not protect aging peoples from sarcopenia (muscle mass loss) (2-3). According to the last edition of L.A.R.N. (Livelli di assunzione giornalieri raccomandati di nutrienti per la popolazione italiana) (4), the protein Average Requirement (AR) in aging is 0,71 g/Kg/die, while the protein Population Reference Intake (PRI) is 0,90 g/Kg/die. Aging is characterized by sarcopenia, a reduction in muscle mass accompanied by frailty and increased risk of disability. However, the actual worldwide trend of increased prevalence of obesity is accompanied by increase in fat mass and decrease in fat free mass, giving origin to Sarcopenic Obesity in aging. Therefore, the addressed question was the nutritional management of sarcopenic obesity in aging. We studied the effect of a diet moderately rich in proteins on lean mass in sarcopenic obese elderly. In 460 patients (328 females), > 65 years old, BMI >30 Kg/m^2, nutritional status was assessed. Sarcopenia was defined in patients according to Muscle Mass Index (MMI) and related cut-off score of 8,5 Kg/m^2 for men and 7,3 Kg/m^2 for women, derived from obese adult population. The patients were treated with hypocaloric diet moderately rich in proteins (1,2-1,3 g/Kg/die) for three months. Among 460 patients (328 women) (age >65 years; BMI=33,9±4,1 Kg/m^2 for men and 35,1±4,6 Kg/m^2 for women), 30 women and 4 men were sarcopenic. After 3 month dieting, in non sarcopenic men (NSM) MMI was 10,2±1,09 kg/m^2 vs 10,4±1,2 kg/m^2, baseline ($p < 0,01$ vs baseline), while in non sarcopenic women (NSW), MMI was 8,4±1,04 kg/m^2 vs 8,6±0,9 kg/m^2, baseline ($p < 0,01$ vs baseline). Sarcopenic groups did not show significant differences in MMI after dieting: in males MMI was 8,6±0,7 kg/m^2 vs 8,1±0,1 kg/m^2, baseline; in females MMI was 7,0±0,5 kg/m^2 vs 6,9±0,2 kg/m^2, baseline. Arm muscular area (AMA) decreased only in non sarcopenic groups, from 63,6±13,6 cm^2, baseline, to 61,3±12,2 cm^2 ($p < 0,001$ vs baseline) in NSM, from 52,2±12,8 kg/m^2, baseline, to 49,9±12,2 cm^2 ($p < 0,001$ vs baseline) in NSW. Sarcopenic groups, conversely, did not show significant differences in AMA. Therefore, in our study, a diet moderately rich in proteins for three months was able to preserve muscle mass only in sarcopenic subjects. In conclusion, adequate protein intake could contribute to prevent lean mass loss in obese aging.

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SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology
Lecture 2

Comparing the regulation of gametogenesis and spawning in bivalve molluscs

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Marine, bivalve molluscs provide a valuable food source and are harvested from environmental sites and through aquaculture. The most used are mussel (*Mytilus spp*) and oyster (*Crassostrea gigas, Ostrea edulis*), but clams (*Mercenaria mercenaria*), scallops (*Aequipecten opercularis*) and abalone (*Haliotis spp*.) are also economically important. They are sessile, sedentary organisms with a wide geographic distribution. Through filter feeding they ingest and accumulate environmental chemicals such as metals, hydrocarbons and pharmaceuticals that may elicit sex-dependent stress responses, also affected by the stage of the reproductive cycle. Aquaculture rearing of molluscs depends on natural reproductive processes which are not well understood. For instance in Mytilus spp the annual cycle consists of gonadal development, maturation, spawning and resting. Ambient temperature is likely a key to the switch from one phase to another, but the identity of other environmental factors and of organism and cellular regulators is far from clear. There is evidence for the involvement of serotoninergic pathways and modulation by prostaglandins. The possible role of steroid hormones has been much discussed but is still not clear. The regulatory mechanisms of reproductive processes demand a better understanding. This presentation will explore what is known about these processes by using a comparative approach covering reports for various species and suggesting ways forward for future investigation.

The role of estrogens in fish immunity

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In mammals, an immunomodulatory role of estrogens is well documented. The actions of estrogens in the immune system are complex; they are mediated through cell type and cell stage-specific distribution of the two estrogen receptor (ER) subtypes of mammals, which induce subtype-specific gene expression profiles, and can act both synergistically and while antagonistically, depending on the immune cell type. For teleost fish, in contrast to mammals, an immunomodulatory role of estrogens has to be demonstrated yet. Recent studies in our laboratory have shown that (i) that treatment of rainbow trout with 17b-estradiol changes the response of immune pathways to pathogen challenge, and increases susceptibility of trout to bacterial infection; (ii) immune organs of rainbow trout express four ER subtypes, ERalpha1, ERalpha2, ERbeta1, and ERbeta2, with expression levels and subtype ratios varying between T-lymphocytes, B-lymphocytes and granulocyte; (iii) immune parameters of rainbow trout undergo changes in relation to endogenous oscillation of circulating estrogen levels, as they occur during the reproductive cycle of mature females. With these findings, we provide the first evidence for the presence of estrogen signaling in teleostean immune cells. Future studies will have to elucidate the target processes that are under estrogen control, and how this translates into alterations of immune capacity of fish.

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Oral Communications

Physiological mechanisms of innate immunity in bivalve molluscs: a lesson from environmental stressors

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Invertebrates, that represent about 95% of animal species and are widespread in all types of environments, lack acquired immunity; however, they are endowed with a potent and complex innate immune system . In invertebrates, innate immunity thus represents one of the main physiological mechanisms to ensure survival in changing environmental conditions. In bivalve molluscs, free circulating cells (hemocytes) are responsible for cell-mediated immunity through phagocytosis, oxidative burst and NO production, release of hydrolytic enzymes and antimicrobial peptides. The physiological mechanisms involved in the immune response have been widely investigated in the marine bivalve *Mytilus*, in response to different environmental stimuli, including bivalve and human pathogens. These studies allowed for the identification of key components of the immune response, the signalling pathways involved, and their modulation by hormones and cytokines, supporting the view that bivalve hemocytes can be considered the invertebrate counterpart of mammalian macrophages. The environmental physiology approach greatly contributed to the identification of the basic mechanisms of innate immunity and to highlight the specificity of the immune response in bivalves. Recent analysis of EST collections and gene function in *Mytilus* confirmed the identity of immune-related genes and underlined their high conservation between bivalves and humans.

Novel mussel haemolymph biomarkers: a system biology approach

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For many years, the haemolymph cells are used to study the mussel stress by evaluating the lysosomal membrane stability and, more recently, lipofuscin and neutral lipid lysosomal accumulation. Moreover, hemocytes are used to study the effects of toxic chemicals on the mollusc immune response. However, little is known about the physiological role of haemolymph serum components i.e. proteins and metabolites. Recently, new haemolymph biomarkers were identified using a proteomic approach. These biomarkers allowed to reveal early warning effects in mussels exposed to increasing temperatures and to sublethal Cu concentrations. A change of the electrophoretic profile of the serum proteins, and in particular of histidine-rich glycoprotein (HRG), was demonstrated. Carbonylation and nitrosylation of serum proteins represent a potential sensitive stress biomarkers. In addition, the first evaluation of the metabolomic profile of mussel serum haemolymph by a NMR approach was realized and 27 metabolites were identified. The changes of the metabolomic profile of the haemolymph serum in mussels exposed to environmental stressors (increasing temperatures and Cu 20 µg/L) revealed that the metabolomic approach may represent an important new tool useful to study in deep some aspects relative to mussel physiology and ecotoxicology.

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Changes in granulocyte cell volume as novel general biomarker of pollutant exposure in invertebrates

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Invertebrate hemolymph and coelomic fluid are interesting tissues from a toxicological perspective for the development of novel non destructive cellular biomarkers. They can transport pollutants throughout the exposed organism and their cellular components are involved in the internal defense system.
The study was aimed to investigate pollutant induced morphometric alterations in invertebrate granulocytes, which are the cell type mainly involved in phagocytosis, in view of future applications as biomarker for environmental monitoring and assessment applications. The study was carried out on four species, *Mytilus galloprovincialis*, *Lumbricus terrestris*, *Eisenia fetida*, and *Cantareus apertus*, by image analysis on Diff-Quick® stained cells.
Granulocyte showed a cell volume increase in organisms exposed to either heavy metals or xenobiotics in all the species considered. The granulocyte enlargement was accompanied by cell rounding with loss of pseudopods. The physiological mechanisms involved in the observed response are discussed. The response observed in laboratory conditions was also validated in field conditions.
In conclusion granulocyte enlargement showed high sensitivity to pollutant exposure with respect to other general standardized biomarkers, and showed several of the necessary characteristics for successful application as an effective biomarker in monitoring and assessment programs.
This study was granted by PRIN project (prot. n. 2010ARBLT7_005).

Parallel Symposia
From molecules to movement:
the impact of key muscle proteins on muscle function and dysfunction

The cellular and molecular mechanisms underlying skeletal muscle adaptations to disuse

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Disuse muscle atrophy is a common event in chronic diseases, which imply a reduced physical activity, in aging and in apparently healthy individuals with sedentary life style. Muscle atrophy becomes in itself a critical condition as it impairs the ability to perform daily tasks, increases the risk of falls and opens the way to chronic, and particularly metabolic, diseases.
In disuse muscle atrophy, key myofibrillar proteins are preferentially lost. Such loss depends on an imbalance between muscle protein synthesis (MPS) and breakdown (MPB). The molecular regulation of MPS and MPB includes the IGF-1/Akt/mTOR pathway, which can enhance MPS, and the Forkhead box O (FoxO) signaling pathway, which can enhance MPB, activating the ubiquitine proteasome system and autophagy. However, the relative contribution of MPS and MPB to disuse muscle atrophy and which factors trigger and support the imbalance between MPS and MPB have not been identified yet.
Oxidative stress (OS) is widely considered a major trigger of MPS and MPB adaptations. OS has been shown to increase the gene expression of key components of proteolysis systems, promote proteolysis in muscle fibers by the oxidative modification of myofibrillar proteins, and inhibit protein translation. However, it is still unclear whether OS is actually a cause or a consequence of disuse atrophy in limb muscles. A comprehensive picture of the pathogenesis of disuse muscle atrophy in a mouse model of disuse (hindlimb unloading) will be provided together with a parallel analysis of a human model of disuse (bed rest).

Parallel Symposia
From molecules to movement:
the impact of key muscle proteins on muscle function and dysfunction

Acetylcholine receptors and Ca^{2+} signals in maturing myotubes

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Membrane depolarization and $[Ca^{2+}]_i$ transients do not uniquely trigger contraction of skeletal muscle fibres, but have wider biological implications at all developmental stages, regulating events such as myoblasts fusion or initiation of apoptotic pathways in disease. Moreover, muscle fibre excitation, indicative of correct nerve-muscle communication, is required for neuromuscular junction maintenance.
Acetylcholine receptors (AChR) are expressed by satellite at the onset of differentiation and are activated in an autocrine loop by an endogenous compound. By this route, AChR orchestrate $[Ca^{2+}]_i$ transients, which in turn regulate myoblast fusion and myotube maturation. IP_3R-mediated Ca^{2+} release also contributes to $[Ca^{2+}]_i$ transients, at least during the early phases of in vitro myotube formation.
Our work aims at understanding the specific role of AChR and IP_3R in the control of $[Ca^{2+}]_i$ and the interplay between the two systems, in particular in human cells. We examine how these pathways work when myogenic potential of satellite cells is reduced, as in aging or disease. To this purpose, we study the formation in vitro of various types of human and mouse myotubes while interfering with $[Ca^{2+}]_i$ transients. We analyze both myoblast fusion and myotube functional maturation, timing the appearance of voltage- and ACh-gated currents. The data collected may help in devising approaches to boost myogenic differentiation when it happens to be impaired.

Parallel Symposia
From molecules to movement:
the impact of key muscle proteins on muscle function and dysfunction

Motor and cytoskeleton proteins of the sarcomere and associated myopathies

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The functional unit of skeletal muscle, the half-sarcomere, produces high power at high efficiency due to the combination of the function of the myosin II array in the myosin filament interdigitating with the actin filament and the meshwork of cytoskeleton proteins acting as a scaffold (Geeves and Holmes, *Adv Prot Chem* **71**:161, 2005; Piazzesi et al. *Cell* **131**:784, 2007). The importance of cytoskeleton proteins is illustrated by the identification of mutations in many of the corresponding human genes in patients with skeletal myopathies. Here we studied the role of the two cytoskeletal proteins nebulin and myopalladin. Nebulin gene mutations are causative for nemaline myopathy while myopalladin gene mutations have been identified in patients with limb girdle muscular dystrophy. To provide insights into the physiological role of these proteins and the mechanisms leading to myopathy, the mechanical performance of whole muscle and/or single fibres from *wt* and *knockout* mice for these proteins is studied. Results indicate that these proteins do not act simply as scaffolds but, as far as sarcomeric structure is preserved, modulate the function of the myosin motors and/or the power output of the muscle. The absence of nebulin decreases the probability of attachment of the myosin motor to actin, while the absence of myopalladin reduces muscle growth without affecting the kinetics of the actomyosin interaction. Supported by Telethon and MIUR.

Parallel Symposia
From molecules to movement:
the impact of key muscle proteins on muscle function and dysfunction

Myopathies and cardiomyopathies associated to sarcomeric protein mutations

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A major focus of our research is to understand mechanisms by which mutations in myofibril proteins cause skeletal and cardiac genetic myopathies. The myofibrils of striated muscle fibres are abundant and highly specialized structures responsible for force-generating contraction and its regulation. Techniques to measure force generation and relaxation of isolated myofibrils in response to sudden changes in [Ca2+] have been developed since some years in our laboratory and are applied to animal and human models of genetic myopathies. In the case of mutations in thin filament regulatory proteins (Tn, Tm) associated to myopathies, replacement of endogenous proteins with recombinant engineered proteins can be used to test in isolated myofibrils which sarcomere phenotype can be rescued by the healthy protein or induced by the mutant protein. Methods to measure sarcomere ATP consumption during isometric force generation are also available in the laboratory to directly measure the energy cost of tension generation in skinned skeletal and cardiac muscle fibres. The impact of some pathogenic mutations in myofibril proteins on skeletal and cardiac sarcomere mechanics and energetics are reported. The results indicate that energy depletion due to inefficient ATP utilization by the sarcomeres may be central to the disease process of different skeletal and cardiac myopathies.

Caveolin-1 expression in lung cells: early sensor of transduction signaling.

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Caveolin-1 (Cav-1) is an integral membrane protein that provides a scaffolding structure essential for the formation of caveolae, a specialized form of lipid microdomain containing signaling proteins, receptors and ion channels. In lung tissue septa, endothelial cells, type I pneumocytes, and fibroblasts display high density of caveolae suggesting their important role in the respiratory system.
In lung pathologies decreased expression of Cav-1 parallels an increase in collagen content. Consistently, Cav-1 KO mice exhibit a decreased lung compliance compared to WT mice, associated with abnormal extracellular matrix deposition, which would likely impact lung mechanics and physiological functions. Considering also that Cav-1 is implicated in the regulation of microvascular permeability, this protein appears to be a critical regulator in lung physiology.
Previous data from our lab showed a differential Cav-1 expression at the air-blood barrier depending on the nature of lung perturbation and a re-distribution of Cav-1 in an *in vitro* model of mild hypoxia.
We will present here the regional distribution of Cav-1 in the lung of normoxic rats, the kinetic of this protein expression in hypoxia and in acute re-oxygenation. We finally propose a role for electric stimulus in Cav-1 distribution/expression in an *in vitro* model of alveolar epithelial cells.
Our findings support the hypothesis of an important role of Cav-1 as an early actor in the mechano-transduction signaling in the lung.

Parallel Symposia
Membrane microdomains and physiological modulation of cellular excitability

An altered interaction of caveolin-3 with cardiac ion channels affects cell excitability

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Caveolins are structural proteins of caveolae whose function is, among others, to facilitate functional interaction among proteins involved in the same signaling pathway. Of the three caveolins known (cav-1, -2 and-3), cav-3 (cav-3) is highly expressed in the heart, both in the working and pacemaker cardiomyocytes. Cardiac action potentials are generated by the precise interplay of several ion channels, transporters and exchangers and even minor alterations in membrane currents may significantly alter cellular excitability. HCN channels, for example, interact with cav-3 through a caveolin-binding domain and disruption of such interaction modify both their basic properties and their modulation by b-adrenergic signaling; this in the end results in a significantly alteration of excitability of sinoatrial node cells. Mutations of the human cav-3 gene cause both skeletal and cardiac diseases. Recently, we have found a cav-3 mutation (T78M) in several patients with inappropriate sinus tachycardia and atrial fibrillation (AF). The evaluation of the functional effect of cav-3 T78M on HCN channels, which are involved in heart rhythm generation, and on Kv1.5 channels, that when altered may trigger AF, revealed a significant gain of function of both channels, which is ultimately compatible with the electrophysiological features of the above pathologies. In conclusion, these data highlight the fundamental role of membrane microdomains in cardiac (patho)physiology.

Modulation of purinergic signalling at the plasma membrane: a multipurpose and multidirectional way to orchestrate physiopathological conditions in the nervous system

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ATP is a signalling molecule copiously released in the extracellular environment upon cell activation, stress, or damage. Extracellular ATP is also a multidirectional information molecule, given the concurrent presence at the plasma membrane of various ectonucleotidases metabolizing ATP, different transporters for ATP/adenosine, fifteen P2 receptors for purine/pyrimidine nucleotides, and four metabotropic P1 receptors for nucleosides. These purinergic proteins often associate with each other generating multiple interactions that overall regulate the insurgence, duration and termination of the purinergic signalling. Specialized sub-membrane compartments as lipid rafts/caveolae are enriched in purinergic proteins and favour proper cooperative behaviour in response to physiopathological requirements. Moreover, endocytic trafficking and exocytosis from lysosomes regulate the constitutive or evoked internalization and recycling of purinergic receptors at the plasma membrane upon agonist binding. In particular, we demonstrated that P2X3, P2Y4, P2Y6 receptors from neuronal cells reside in lipid rafts, and that endocytosis and targeting of P2X3 to degradative pathways as late endosomes/lysosomes is ligand-induced, cholesterol-dependent, but chlatrin- and autocrine ATP release-independent. Although the implications for receptor functional regulation in vivo are still premature, our findings of the dynamic trafficking of P2X3 disclose a mechanism for the rapid modulation of ATP-mediated responses potentially relevant during physiopathological conditions in the CNS.

Parallel Symposia
Membrane microdomains and physiological modulation of cellular excitability

Caveolinopathies: translational implications of caveolin-3 in skeletal muscle disorders

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In skeletal muscle the protein Caveolin-3 forms caveolae, flask-shaped invaginations localized on the cytoplasmic surface of the sarcolemmal membrane. Caveolae play a key role in the maintenance of plasma membrane integrity, in vesicular trafficking and signal transduction. Preservation of physiological Caveolin-3 levels is essential for normal muscle development and for postnatal skeletal muscle function. Mutations in Caveolin-3 gene lead to muscle pathology through multiple mechanisms: sarcolemmal membrane alterations, disorganization of T-tubule network and disruption of distinct cell-signaling pathways. Caveolin-3 defects are associated to four distinct skeletal muscle disease phenotypes: limb girdle muscular dystrophy, rippling muscle disease, distal myopathy, and asymptomatic hyperCKemia. Many patients show an overlap of these symptoms and the same mutation can be linked to different clinical phenotypes, suggesting the occurrence of additional genetic or environmental factors. Until recently, the research on caveolae biology has focused almost exclusively on caveolins and their translational implications. However, further studies have highlighted the existence of a new additional step of regulation, unveiling the role of a family of proteins, the cavins, which seem to play a critical role in caveolar formation and function. In the cytosol, cavins form a complex, that is recruited to membrane caveolae with which cavins establish mutually stabilizing interactions.

Hemoglobins: models of physiological adaptation, with special reference to O_2 availability and temperature

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In transporting O_2 from the respiratory surfaces to the respiring tissues of animals, hemoglobin (Hb) directly links aerobic metabolism with O_2 availability and is a paradigm for studying mechanisms of molecular adaptations. Hb-O_2 binding is cooperative (described by sigmoid O2 binding curves) and decreased by allosteric effectors (protons, CO_2, lactate, organic phosphates and chloride ions) that modulate O_2 binding in response to changes in environmental and metabolic dictates. Hb-O_2 affinity moreover decreases with rising temperature. This increases O_2 unloading in warm tissues that consume more O_2, but may be maladaptive – and thus is reduced - in regional heterothermic animals where it may hamper O_2 unloading (in cold extremities of Artic mammals) or cause excessive O_2 release (in warm organs of fast-swimming fish). Illustrated with case studies (estivating lungfish, high altitude frogs, birds and mammals - and recreated woolly mammoth Hb) the treatise reviews *intr*aspecific and *inter*specific adaptations (that are mediated by changes in the levels of red cell effectors and in Hb structure, respectively) in response to changes in O_2 availability and temperature, demonstrating reciprocity of compensatory adjustments at molecular, cellular and organismic levels of organization.

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Oral Communications

Entrapment of hemocyanin conformers as a tool for the definition of the structural model of cooperativity

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Oxygen fluctuations in marine environment represent one of the major stress for marine organisms. To satisfy the oxygen demand, many physiological compensation mechanisms have been evolved to maintain homeostatic balance of oxygen in body tissues. The hemocyanins, the oxygen transport proteins, play an important role in the regulation of the oxygen homeostasis in many arthropods and molluscs. Despite the thorough phenomenological descriptions of the hemocyanins oxygen binding behavior, the structural basis of the changes in oxygen affinity in dependence of the oxygen concentration are still unclear. To this aim, we defined a structural model of cooperativity for *Carcinus aestuarii* hemocyanin, through the encapsulation of the protein into a sol-gel matrix that allows the functional and structural characterization of different conformational states. We propose a mechanism to describe how the quaternary conformational changes are linked to the different coordination geometry of the active site during the oxygen binding process. The functional and structural effects of the allosteric effectors H^+ and lactate were also characterized at the levels of distinct protein conformers. The results show that the oxygen binding process of *C. aesturaii* hemocyanin is adequately described by the three-state MWC model, with the T-, S- and R- states characterized by different coordination geometry in the deoxygenated form. From a physiological point of view, the presence of three distinct conformers increase the functional plasticity of the hemocyanin in response to different environmental conditions.

Environmental stress and nitrogen excretion in freshwater teleosts

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Environmental stress conditions can alter nitrogen excretion of freshwater fish. Here, we present a series of experimental data from zebrafish (*Danio rerio*), gambusia (*Gambusia affinis*), goldfish (*Carassius auratus*) and convict cichlid (*Amatitlania nigrofasciata*), which demonstrate changes in arginase activity and urea excretion following different acute stress treatments (including high salinity, temperature, environmental nitrite and hypoxia/reoxygenation). These data suggest that the tolerance of the animals to environmental changes could be related with changes in the arginine-NO homeostasis. This possibility was tested on zebrafish under acute hypoxia/reoxygenation stress (H/R). Reoxygenation can be even more stressful than hypoxia, mainly because of the excess ROS production of mitochondrial respiratory chain, and the consequent alteration of redox state. Experiments have been performed on the effects of H/R on nitrogen excretion, arginase activity, nitrite tissue levels, muscle mitochondrial properties, muscle antioxidant defenses, and NOS and arginase mRNA expression in different tissues (gills, liver/intestine and muscle). The results indicate that the ability of zebrafish to face H/R is related with both the characteristics of muscle mitochondria and redox state, and the expression of NOS II and arginase.

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The physiological urination mechanism at hypoxic altitudes

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The chronic exposure to hypoxia results in an overactive sympathetic nervous system that causes relaxation of the bladder body, its neck contracture along with contraction of the proximal urethral portion. The aim of this study was to determine possible correlation between changes in pO_2 and the physiological functionality of vesicourethral unit, to identify any physiological role of hypoxia in the mechanism of urination. The study was carried out on 7 females (27-48 years old). To assess variations of uroflowmetry parameters, a 2002 Microflo II Model was used, at sea level before and after the expedition (SEAPRE and SEAPOST), at 3500 (HIP3500) and at 5000 m (HIP5000), in the Pyramid Laboratory Nepal managed by the Ev-K2-CNR group. The evaluated urinary flow parameters were: maximal and mean flow rate, time to maximal flow, flow and voiding time, flow volume. The maximal and mean flow rate and time to maximal flow showed no significant changes in percentage variation (Δ%). The flow volume Δ% was significantly increased in HIP5000 m compared to SEAPRE. The flow time Δ% was significantly increased in HIP5000 compared to SEAPRE. Significant decrease was seen for SEAPOST compared to HIP3500 and HIP5000. The voiding time Δ% was significantly increased in HIP5000 versus SEAPRE. Significance $p < 0.05$.Our experimental system for altitude hypoxia has provided a first *in-vivo* model of alterations in the physiological micturition reflex related to the reduction of the breathed pO_2.

Environmental challenges and organ morpho-functional remodeling: the African lungfish as a case study

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African lungfishes (*Protopterus* spp.) are obligate air breathers that, during the dry season, experience a prolonged torpor (aestivation) associated with water and food deprivation, deep metabolic depression and profound biochemical and morpho-functional readjustments at tissue and organ level. During aestivation respiratory organs undergo significant rearrangements. Gills became non-functional with consequent complete reliance on lung breathing. Renal excretion is reduced, the heart continues to pump, although at low frequencies, and the skeletal muscle stops to function, being able to immediately contract during arousal.
So far, few studies have investigated the multilevel changes occurring in lungfish during the transition from aestivation and arousal, and *vice versa*. We recently demonstrated that a crucial role in the orchestration of these environmental stress-induced adaptations is played by the ubiquitary gasotransmitter nitric oxide (NO). By studying two lungfish species (*P. dolloi* and *P. annectens*), we found that the eNOS/NO system is implicated in the morpho-functional readjustments occurring at the level of kidney, heart and skeletal muscle. These changes are accompanied by modifications of cell turnover in terms of apoptosis. Our studies are the first to reveal molecular aspects of the complex mechanisms which sustain lungfish adaptation to environmental stressful challenges, allowing not only survival but also a rapid switch from aestivating to freshwater conditions.

SIF-ESCPBnew WORKSHOP on Comparative and Environmental Physiology
Oral Communications

A protein cold adaptation strategy *via* a unique seven amino acid domain in the icefish (*Chionodraco hamatus*) PEPT1 transporter

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Adaptation of organisms to extreme environments requires proteins to work at thermodynamically unfavorable conditions. To adapt to subzero temperatures, proteins increase the flexibility of parts or even the whole three-dimensional structure to compensate for the lower thermal kinetic energy available at low temperatures. This may be achieved through single site amino acid substitutions in regions of the protein that undergo large movements during the catalytic cycle such as in enzymes or transporter proteins. Other strategies of cold adaptation involving changes in the primary amino acid sequence have not been documented yet. In the Antarctic icefish (*Chionodraco hamatus*) peptide transporter PEPT1, the first transporter cloned from a vertebrate living at subzero temperatures, we discovered a novel principle of cold adaptation. A *de novo* domain composed of one to six repeats of seven amino acids (VDMSRKS), placed as an extra-stretch in the cytosolic COOH-terminal region, contributed *per se* to cold adaptation. VDMSRKS was in a protein region uninvolved in transport activity, and notably, when transferred to the COOH-terminus of a warm-adapted (rabbit) PEPT1, it conferred cold-adaptation to the receiving protein. Overall, we provide a new paradigm for protein cold adaptation that relies on insertion of a unique domain that confers greater affinity and maximal transport rates at low temperatures. Due to its ability to transfer a thermal trait, the VDMSRKS domain represents a useful tool for future cell biology or biotechnological applications.

Isolating sinoatrial precursors from embryonic stem cells for the development of a biological pacemaker

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The sinoatrial node (SAN) is the natural pacemaker of the heart and its dysfunction may cause serious pathologies usually treated by implanting an electronic pacemaker. Recently, researchers attempted to develop a biological pacemaker, that is a cellular substrate able to induce ectopic spontaneous activity in the host tissue. A promising strategy could be based on differentiation of embryonic stem cells (ESCs) and selection of the population with functional properties of SAN myocytes, however it has been hampered so far by the lack of proper markers.
We succeeded to select a population of pacemaker precursors based on CD166 expression, at day 8 of mouse ESC differentiation. This protein is transiently co-expressed with HCN4, a marker of the cardiac pacemaker tissue, during mouse heart development. In culture, CD166-selected cells develop into spontaneously beating cells that express high levels of typical SAN genes and can form an autorhythmic syncytium with the molecular and electrophysiological properties of mouse SAN myocytes. Firing rate of CD166-selected cells can be modulated by autonomic neurotransmitters. Furthermore, in an *in vitro* co-culture system, CD166-selected cells are able to connect to and pace newborn ventricular myocytes at a faster rate than their own, thus behaving like a real pacemaker.
Our results open a new perspective in the generation of biological pacemakers, based on human pluripotent stem cells, for future clinical applications.

Parallel Oral Communication
Cell Physiology

Functional involvement of β3-adrenergic receptors and nitric oxide in melanoma growth

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β-adrenergic signaling facilitates cancer progression and blockade of β-adrenergic receptors (β-ARs) slow down tumor growth. A role of β3-ARs in tumor growth has not been investigated yet. In this study, we investigated the involvement of β3-ARs in melanoma growth and the possible involvement of nitric oxide (NO) as a downstream β3-AR effector. Both *in vitro* (cultured B16F10 melanoma cells) and *in vivo* (mice bearing syngeneic B16F10 cells) models were used. The data showed that β3-ARs are expressed in mouse B16F10 cells and that β3-AR blockade with SR59230A or L-748,337 reduce cell proliferation and induce apoptosis. In particular, *in vivo* intra-tumor injections of SR59230A or L-748,337 resulted in significant decrease of the tumor vasculature due to apoptosis of endothelial cells. These effects were likely to be mediated by modulation of NO production. Indeed, NO donors or NO synthase (NOS) activation prevented the effects of β3-AR blockade on cell proliferation and apoptosis. In addition, NOS inhibition replicated the effects of β3-AR blockade. This study shows that reducing β3-AR function is an effective way of contrasting melanoma growth. Our results also demonstrate that the NO pathway is involved in β3-AR signal transduction, indicating NO as a possible molecular switch between cell proliferation and death. Together, these findings indicate β3-ARs and their downstream effectors as promising, novel targets for anti-cancer therapy.

A novel human Aquaporin-4 splice variant exhibits a dominant-negative activity: a new mechanism to regulate water permeability

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Two major isoforms of Aquaporin-4 (AQP4) have been described in human tissue. Here we report the identification and functional analysis of an alternatively spliced transcript of human AQP4, AQP4-Δ4, that lacks exon 4. In transfected cells AQP4-Δ4 is mainly retained in the ER and shows no water transport properties. When AQP4-Δ4 is transfected into cells stably expressing functional AQP4, the surface expression of the full-length protein is reduced. Furthermore, the water transport activity of the co-transfectants is diminished in comparison to transfectants expressing only AQP4. The observed down-regulation of both the expression and water channel activity of AQP4 is likely to originate from a dominant-negative effect caused by heterodimerization between AQP4 and AQP4-Δ4, which was detected in co-immunoprecipitation studies. In skeletal muscles, AQP4-Δ4 mRNA expression inversely correlates with the level of AQP4 protein and and is physiologically associated with different types of skeletal muscles. To our knowledge this is the first example of a potential regulatory mechanism through which the cell-surface expression and activity of AQP4 can be physiologically modulated.

Parallel Oral Communication
Cell Physiology

Adaptative cellular responses to hypoxia: role of HIF-1 dependent genes in endothelial cell autophagy and survival

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Hypoxia, or a decreased partial oxygen tension (pO_2), occurs in many physiological processes, including angiogenesis. Hypoxia can have dual consequences depending on the cellular context and on the mechanisms that enable the cells to adapt to changes in the pO_2, inducing cell survival or apoptosis. HIF-1 is the master regulator of the adaptive responses to hypoxia and its α subunit is able to interact with hypoxia response elements to induce transcriptional activity, resulting in the expression of a variety of genes, such as the vascular endothelial growth factor (VEGF) and BCL2/adenovirus E1B 19kDa interacting protein 3 (BNIP3). We have previously shown that HIF-1α is involved in cell survival and/or apoptosis in different cell types, including immune and tumour cells. Despite the fact that hypoxia represents a microenvironmental stress, several reports indicate that endothelial cells are especially adept at surviving under hypoxic conditions and that autophagy of endothelial cells promotes angiogenesis. We here report that hypoxia enhances the expression of BNIP3, a molecule that is crucial for hypoxia-induced autophagy, and increases the amount of the smaller-molecular-weight LC3-II protein, a hallmark of autophagy, in human umbilical vein endothelial cells (HUVEC). More interestingly, hypoxic endothelial cells isolated from patients affected by pathologies, constantly characterised by angiogenesis, expressed a higher level of BNIP3 and a lower Bax/Bcl2 ratio, when compared with HUVEC. These results underline the central role played by HIF-1α/BNIP3 axis in the autophagic process and, thereby, in endothelial cell survival.

Post-conditioning with catestatin (CST-Post) protects the heart of spontaneously hypertensive rats (SHR) from ischemia/reperfusion injury and triggers anti-apoptotic and pro-angiogenetic factors

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Post-conditioning protects heart from reperfusion injury *via* RISK-pathway; however in the presence of comorbidities the effectiveness of protective strategies is blunted. Here, we studied in isolated hearts of a hypertensive rat model (SHR) whether anti-hypertensive and pro-angiogenic chromogranin A peptide CST infusion in early reperfusion (CST-Post) protects the heart *via* RISK pathway activation, limiting infarct size (IS), apoptosis and promoting angiogenetic factors. The hearts underwent the following protocols: (a) 30-min ischemia and 120-min reperfusion (I/R); (b) 30-min ischemia and 20-min reperfusion (I/R-short), both with and without CST-Post (75nM for 20-min at the beginning of reperfusion). IS was also studied in Wistar-Kyoto control hearts where IS resulted smaller than in SHR. CST-Post reduced significantly IS in both strains. After 20-min reperfusion, CST-Post induced phosphorylation of RISK elements, and inhibitors of the RISK pathway blocked the CST-Post protective effects against IS in the 120-min reperfusion groups. Moreover, apoptosis (TUNNEL and ARC) was reduced by CST coupled with increased expression of pro-angiogenetic factors (*i.e.*, HIF-1α and eNOS) after two-hour reperfusion. In conclusion CST-Post limits reperfusion injury and reverses the hypertension-induced increase of I/R susceptibility. Moreover, CST-Post triggers antiapoptotic and pro-angiogenetic factors suggesting that CST-Post can be used as an anti-maladaptive remodeling treatment.

Parallel Oral Communication
Cell Physiology

Calsequestrin-1 in skeletal muscle: what we learned from knockout animals

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Calsequestrin type 1 (CASQ1), the main sarcoplasmic reticulum (SR) Ca^{2+} binding protein, plays a dual role in skeletal fibers: a) it provides a large pool of rapidly-releasable Ca^{2+} during excitation-contraction (EC) coupling; and b) it modulates the activity of ryanodine receptors (RYRs), the SR Ca^{2+} release channel. We generated a mouse lacking CASQ1 (CASQ1-null) in order to further characterize the role of CASQ1 in skeletal muscle. Contrary to initial expectations, CASQ1 ablation is compatible with normal motor activity, in spite of moderate muscle atrophy. However, CASQ1 deficiency results in profound structural remodeling of the EC coupling apparatus and significant functional impairment: prolonged time course of contraction, reduced size of Ca^{2+} transients, increased rate SR Ca^{2+} depletion, and inability to sustain tension during a prolonged tetani. To confirm that such changes are directly caused by CASQ1 ablation (and do not result from adaptive mechanisms), we attempted rescue of the null phenotype by cDNA electro transfer in flexor digitorum brevis muscle. Exogenous CASQ1 is found to be correctly targeted to the junctional SR (jSR), as judged by confocal and electron microscopy; peak amplitude of Ca^{2+} transients is significantly increased; and transfected fibers are now able to sustain cytosolic Ca^{2+} concentration and tension during prolonged tetanic stimulation, all indication that changes in knockout mice were directly caused by ablation of CASQ1.

The role of S6 kinase in the regulation of skeletal muscle mass and function

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The kinase Akt is a very important regulator of skeletal muscle mass, with its activation stimulating protein synthesis and blocking protein degradation leading to muscle hypertrophy. Akt has numerous downstream effectors which might contribute to increase muscle mass, amongst which mTORC1 and S6K1. In this project we want to examine through which pathways activation of Akt leads to hypertrophy and improved adult muscle function in vivo, focusing on S6K1 and S6K2.
In preliminary experiments, we transfected a plasmid coding for a constitutively active Akt into adult S6K1 k.o. mice, measured fiber size and compared this to the hypertrophy seen after transfection in wildtype animals. We found that Akt induces a similar hypertrophy in tranfected fibers from k.o. mice as compared to wildtype animals. In order to better understand which function S6K1 fullfills in skeletal muscle hypertrophy, we have generated a new transgenic mouse line by crossing a transgenic line in which Akt can be inducibly expressed in skeletal muscle, with the S6K1 k.o. line, leading to the generation of the Akt-S6K1 k.o. Activation of Akt in Akt-S6K1 k.o. for three weeks leads to an almost two-fold increase in fibersize. Normalized muscle force however is significantly decreased and hypertrophic muscles show areas of muscle degeneration. Taken together this work shows that S6K1 is not required for increasing muscle mass, but is fundamental for increasing muscle force.

Parallel Oral Communication
Physiology of Motor Systems and Exercise

Effect of high-intensity-interval-training (HIT) on maximal aerobic power and ventilatory threshold in older adults

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We tested if 8 weeks of HIT can induce a significant increase of $V'O_{2max}$ and of the $V'O_2$ corresponding to gas exchange threshold (GET) and to respiratory compensation point (RCP) in older men. To this aim, we measured in 12 healthy male volunteers (68.7±3.9 yy; 79.0±10.8 kg, 171.4±5.4 cm) $V'O_{2max}$, GET and RCP before (PRE) and after (POST) 8 weeks of HIT performed 3 times a week cycling 7 times for 2 minutes, interspersed with 2 minutes of recovery, at about 85-90 % of $V'O_{2max}$. GET was measured during an incremental test up to the limit of individual tolerance. $V'O_{2max}$ was measured during a subsequent constant–workload test performed at 105 % the maximal work-rate achieved on the ramp test. Absolute and relative $V'O_{2max}$ significantly increased by 5.4 % (PRE: 2.34±0.32 l/min; POST: 2.48±0.29 l/min, $p < 0.05$, effect size (ES) = 0.7) and 11.7 % (PRE: 28.8±5.66 ml/min kg; POST: 32.6±5.66 ml/min kg, $p < 0.05$, ES = 0.8), respectively. $V'O_2$ at GET and RCP increased by 7.2% (PRE: 17.0±2.86 ml/min kg; POST: 18.3±3.81 ml/min kg, $p < 0.05$, ES = 0.7) and 15.4 % (PRE: 22.8±3.75 ml/min kg; POST: 27.0±5.30 ml/min kg, $p < 0.05$, ES = 1.3), respectively. Moreover, RCP increased from 76.5 % of $V'O_{2max}$ to 82.9 % of $V'O_{2max}$ ($p < 0.05$, ES = 0.7). It is concluded that 8 weeks of HIT are able to induce significant increases of $V'O_{2max}$ and of exercise resistance in older adults.

The ischemic block of the forearm abolishes index-finger's movements but not its associated APAs

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The voluntary movement induces postural perturbations which are counteracted by unconscious feed-forward motor activities, known as anticipatory postural adjustments (APAs). Thus, for every movement, two motor commands are dispatched: one recruiting the prime mover and one driving APAs. These commands are classically thought to be separately controlled, this study investigates if they could be instead considered as a single element of the motor program.
We analyzed APAs in Biceps Brachii, Triceps Brachii and Anterior Deltoid that precede an index-finger flexion (Flexor Digitorum Superficialis). APAs and prime mover activation were recorded in three conditions: before, during and after a long lasting ischemic block of the forearm. Ischemia suppressed both the forearm sensory feed-back and the prime mover activation, thus canceling finger flexion and the ensuing postural perturbation. Thus, a suppression of APAs should be expected, since purposeless and uneconomical. Intriguingly enough, large APAs were instead apparent without significant differences in latency and amplitude, except for Anterior Deltoid that showed smaller APAs during ischemia.
The observation that APAs remain tailored to the intended movement in absence of perturbation, supports the idea that postural and voluntary commands cannot be separated each other, as they both belong to an unique motor program.

Parallel Oral Communication
Physiology of Motor Systems and Exercise

Arm reaching movements modulate action-related verbs free recall: an embodied memory account

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Several studies suggest that the processing of action-related verbs' semantics relies on an internal enactment of the sensory-motor experience associated with the verb. Thus the understanding of a word such as to grasp would require a somatotopic activation of the motor schema underlying the execution of the same act. We hypothesized that this might also affect the retrieval of action-related verbs from memory. 30 healthy participants performed two go/no-go tasks in counterbalanced order. In the semantic task subjects had to execute arm reaching movements towards a peripheral target when they read action-related verbs and to refrain from moving when they read abstract verbs. In the color discrimination task we presented the same verbs, but half of the times printed in green and in the other half printed in red. Subjects had to touch the peripheral target when the verbs were green and to refrain from moving they were red. After the first experimental session, subjects underwent a surprise free recall test, in which they had to recall as many verbs as they could. We found that when subjects were required to make semantic judgments, reaction times were longer after reading hand-related verbs (e.g., to cut). Importantly, the free recall of hand-related verbs was impaired with respect to foot-related one. Our results indirectly showed motor system's recruitment during the processing and retrieval of action-related verbs, strongly supporting the embodied theory of language.

O_2 cost of cycling, respiratory muscles training and exercise tolerance in obese adolescents

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In obese adolescents (OB) an increased work of breathing can negatively affect exercise tolerance. The purpose of the study was to determine whether, in OB, an endurance training program of respiratory muscles (RMT) can decrease the O_2 cost of exercise and enhance exercise tolerance. Before and after 3 weeks of RMT (5 days/wk, isocapnic hyperpnea), nine male OB (age 16.0±1.4 [x±SD] years, body mass 114.4±22.3 kg, body mass index 39.0±7.5 kg/m^2) performed, on a cycle ergometer: an incremental exercise to voluntary exhaustion; a 12-min constant work-rate exercise (CWR) at 120% of the gas-exchange threshold determined before RMT. Pulmonary O_2 uptake ($V'O_2$) was measured breath-by-breath. RMT did not significantly affect peak $V'O_2$. During CWR: the amplitude of the fundamental component of $V'O_2$ kinetics was lower after (1.54±0.44 L/min) vs. before (1.66±0.41) RMT; the slow component was abolished or its amplitude was markedly reduced after RMT (4.4±5.4% of the total amplitude of $V'O_2$ response) vs. before RMT (8.9±4.5). The O_2 cost of cycling was lower after (13.0±1.8 mL/min/watt) vs. before RMT (14.8±1.7). Rates of perceived exertion for dyspnea/respiratory discomfort and for "leg effort" were lower in after vs. before RMT: 3.6±2.1 vs. 5.2±2.4 and 4.4±2.4 vs. 6.1±2.9, respectively. By lowering the O_2 cost of cycling, RMT can significantly enhance exercise tolerance during submaximal exercise in obese adolescents.
Progetti di Ricerca Corrente, Istituto Auxologico Italiano, Milano.

Parallel Oral Communication
Physiology of Motor Systems and Exercise

Toward the realization of a sarcomere like machine: force spectroscopy of an ensemble of motors (HMM) from myosin II of frog skeletal muscle

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We report a preliminary stage toward the realisation of a synthetic sarcomere like machine (MYOMAC) consisting of a single actin filament interacting with an array of motor proteins regularly distributed on an inorganic nano-structured surface. A Dual Laser Optical Tweezers system (DLOT, range 0.5-200 pN force, and 1-10,000 nm displacement) has been setup to measure the mechanical output of the bio-machine under either length or force control. The correct polarity of the actin filament (5-15 μm long) is controlled by attaching its barbed end to a trapped bead *via* gelsolin protein. The protocols for the preparation of myosin II from frog skeletal muscle (Elangovan et al., *J. Physiol.* **590**:1227-1242, 2011) have been further refined to produce its proteolytic fragments, HMM and Subfragment-1 (S1). Mechanical measurements have been done with a simplified version of MYOMAC, where the motor proteins (HMM) are randomly adsorbed on the flat tip of an etched optical fibre (diameter ~5 μm), the position of which is controlled by a nano-piezoelectric actuator. When the actin filament is pulled away in the direction perpendicular to the motor deposited surface, the rupture events show that the rupture force of a single actin-HMM bond is 12.85 ± 0.35 pN. Alternatively, when a steady force of ~8 pN is imposed on the motors, the lifetimes of the actin-HMM bonds show a time constant of ~1 s, in agreement with the value reported for mammalian myosin S1. Supported by IIT, SEED – MYOMAC, Genova.

Cerebellar integration in cortical sensorimotor circuits as a substrate for motor coordination plasticity

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The sense of touch is used by all animals to build a spatial representation of the surrounding environment and to adapt their behaviour consequently. A large fraction of the tactile information is acquired in a dynamic context, while the body is moving in space and brought to contact with the environment either incidentally or purposely. The nervous system must thus combine the sensory and motor informations to optimize movements aimed at collecting information and correctly interpret the sensations. How and where this process takes place in the brain is still largely unknown. In this work we analyzed cerebellar contribution to sensorimotor integration in the whisker system of mice. We identified a new area in the cerebellum where cortical sensory and motor inputs converge at the cellular level. Optogenetic stimulation of this area triggers motor cortex activation and alters parameters of ongoing movements. These results shed a new light on the cerebellum as an active component of sensorimotor circuits and show the functional importance of sensorimotor cortico-cerebellar loops in the dynamic control of voluntary movements.

Parallel Symposia
Neural plasticity in health and disease: insights from animal models

Entorhinal cortex as a model to study synaptic plasticity in neurodegeneration

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Entorhinal cortex (EC) is a parahippocampal region involved in learning and memory and exhibiting an high degree of plasticity. The EC is an important locus of information exchange as superficial layers receive projections from association areas via the perirhinal cortex and then provide the majority of inputs to the hippocampus. Using an in vitro slice preparation, basic properties of synaptic transmission and long-term forms of synaptic plasticity, such as LTP and LTD, have been characterized in EC superficial layer. As EC is early affected during Alzheimer's disease (AD), a particular attention has been dedicated to the impact of beta-amyloid (Aβ) on EC synaptic function, using either exogenous supply or transgenic mice carrying human mutations of APP (mhAPP). The results indicate that increasing concentrations of Aβ causes progressive synaptic impairment as LTP is affected first whereas LTD and synaptic transmission are disrupted by higher Aβ levels. These results were confirmed in mhAPP mice, which display progressive plasticity impairment in EC that precede the synaptic deficits in the hippocampus. These results suggest an exact order of involvement of different circuitries in the synaptic failure underlying Aβ accumulation. Although an in vitro system cannot recapitulate the complexity of AD pathology, the slice model allowed to identify the precise synaptic mechanism and the molecular pathways that could be involved in plastic changes associated to neurodegeneration.

Determinants of impaired hippocampal plasticity in experimental models of neurodegenerative diseases

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Because of its fundamental role in learning and memory, hippocampus exhibits high structural and functional plasticity that includes adult neurogenesis in the dentate gyrus, synaptogenesis in the CA1 region, dendritic remodeling in the CA3 region and modulation of the synaptic strength. The cognitive decline observed in Alzheimer's Disease (AD), the most common neurodegenerative disorder of the elderly, is associated to accumulation of amyloid-β protein (Aβ) oligomers in the hippocampus and brain cortex. Memory loss correlates with A β load and impaired hippocampal plasticity as reflected by reduced long-term potentiation, dendritic spine remodeling, and synaptic protein expression.
By molecular, structural and functional analyses we assessed the relative contributions of intracellular and extracellular Aβ accumulation to hippocampal plasticity impairment. In particular, we demonstrated that i) Aβ buildup in neurons disrupts synaptic function by a mechanism involving caspase-3 activation, and ii) the synaptotoxicity of extracellular Aβ at least partly depends on its internalization in neurons. Moreover, increased levels of intraneuronal Aβ negatively affect proliferation and neuronal differentiation of hippocampal neural stem cells. In summary, our studies shed new light on the mechanisms responsible for the hippocampal plasticity failure that underlies the Aβ-mediated cognitive impairment observed in AD sufferers.

Parallel Symposia
Neural plasticity in health and disease: insights from animal models

Environmental therapy for plasticity enhancement in a Down Syndrome model

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Neural plasticity refers to the remarkable property of neurons to modify their structure and function in response to experience, a fundamental theoretical theme with major implications for neural rehabilitation. We recently demonstrated that two different experimental approaches, namely environmental enrichment (EE), consisting in an enhanced cognitive, sensory, social and motor stimulation, and chronic treatment with fluoxetine, have a dramatic impact on adult brain plasticity, promoting a reopening of high plasticity windows similar to those characterizing early critical periods. A common feature of these paradigms is their ability to reduce the cerebral inhibition/excitation balance, one major regulator of neuronal plasticity. Based on these premises, we further investigated whether EE and treatment with fluoxetine can favor substantial functional recovery in the Ts65Dn mouse line, the most widely studied animal model of Down syndrome (DS), a genetic condition characterized by excessive levels of inhibition in the brain. Our results show that EE and fluoxetine administration can both favor a marked recovery of learning and memory abilities and hippocampal synaptic plasticity, by acting through a reduction of GABAergic inhibition levels. These results encourage the application of intervention protocols based on enriched experience and/or fluoxetine administration in neurological disorders characterized by a dysregulation of the brain inhibition/excitation balance.

Parallel Symposia
Update in respiratory translational medicine:
from alveoli to the whole lung and from experimental model to humans

Alveolar mechanics studied by in-vivo microscopy

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A new methodology based on in-vivo microscopic imaging (60X) of alveoli through the intact pleura was proposed to investigate their mechanical behavior during unrestrained lung movement.
Alveolar morphology was evaluated in anesthetized and paralyzed rabbits through a custom software to obtain the relationships between distending pressure versus alveolar area, septal thickness and alveolar volume density (AVD). On the average, AVD increased 1.4 times on increasing lung distending pressure up to 17 cmH_2O, while septal thickness decreased by a factor of 0.63. The skewness of the distribution of morphological parameters was discussed in terms of regional differences of alveolar absolute and specific compliance as related to alveolar size at functional residual capacity.

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Regional impact of mechanical ventilation and fluid load on lung parenchyma

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The impact of various combinations of mechanical ventilatory strategies and fluid load on the glycasaminoglycan composition of the ventral/top and dorsal/bottom lung tissue has been studied in anesthetized supine rats mechanically ventilated for 4 hours in air: a) at low (~ 7.5 ml/kg) or high (~ 23 ml/kg) tidal volume (V_T) and 0 cmH_2O alveolar pressure; b) at low or high V_T at 5 cmH_2O positive end-expiratory pressure (PEEP) and; c) with or without 7 ml/(kg·h) intravenous saline infusion. Data indicate that even a relative short period of mechanical ventilation tends to degrade the lung extracellular matrix, with alveolar septa thinning and structural GAGs disorganization. Regional tissue damage depends upon V_t, interesting the entire parenchyma at low V_t ventilation, but only the dorsal/bottom region at high V_t. The protective effect of PEEP against the damaging action of stress and strain was evident mainly in the ventral/top lung. Intravascular fluid load recruited the previously poorly perfused ventral/top lung microvasculature, extending the lesional effect of increased shear stress to the endothelial wall of this region. Hence, our data suggest, even in small animals whose size does not allow to clearly empathize regional lung differences, that the extracellular matrix of the ventral/top region is more susceptible than the dorsal/bottom one to the mechanical/hydrodynamic stresses encountered by the tissue during mechanical ventilation and intravascular fluid load.

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Lung tissue matrix remodeling: correlation with pathophysiology of lung edema in normal and mechanically ventilated lung: mechanisms of lung cellular response to the need for interstitial matrix remodeling

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Matrix remodeling is a key factor to assure the functional integrity of the lung alveolo-capillary unit in control conditions as well as when perturbations of the steady state occur. We are interested in the decision sequence of the lung cells to face various needs to maintain homeostasis and an efficient gas diffusion. We consider the role of specific platforms of the plasma membrane, known as caveolae and lipid rafts, that can favor a rapid adaptive response to perturbations as they can act as signaling platforms and furthermore host packages of ready- to-act enzymes involved in matrix remodeling. Recent studies reveal considerable regional differences in the adaptive response to edemagenic conditions. We report here lung regional expression values in control conditions for matrix remodeling enzymes (MMP2 and MMP17, hosted in caveolae and lipid raft, respectively), KGF (a growth factor controlling the deposition of proteoglycans), PGC1α (a cellular oxygen sensor involved in mitochondrial biogenesis) and VEGF (an endothelial Growth Factor). We also analyzed how these expressions are affected by hypoxia exposure and recovery to normoxia.

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Functional imaging for the assessment of regional ventilation in health and emphysema

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Over the past 25 years, x-ray computed tomography (CT) has emerged as a method that can provide an integrated view of both structure and function on a regional basis of the lung. Techniques such as MRI with polarized gases, proton MRI and Xe-enhanced CT in conjunction with high-resolution computed tomography (CT) offer unique, complementary information and are expected to offer enhancements to the knowledge base of the normal and emphysematous human lung. Recently, standard CT has been increasingly considered not only to study parenchymal and airway wall anatomical alterations in emphysema but also to provide data on regional lung function by using images acquired at different lung volumes. More specifically, we have recently demonstrated how the analysis of the variations of specific gas volume (SV_g), i.e. the volume of gas per gram of lung tissue, between different lung volumes (at least two) can provide a valuable tool for clearly identifying and quantifying the extent and severity of trapped gas in the lung. The originality of the proposed methodological approach will have important clinical and physiological implications for surgical planning and evaluation after intervention and also for the assessment of different stages of the disease, and in the evaluation of pharmacological treatment.

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Effects of hydrothorax and lobar resection on lung mechanics

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We evaluated in animal model the effect of individual and combined action of lung resection and hydrothorax on lung compliance. Experiments were done in anesthetized, mechanically ventilated rabbits (2÷2.2 Kg) randomized in two groups: 1) experimental hydrothorax (2 to 8 ml) (N=5), 2) right lower lobe lobectomy (N= 4) and right middle plus lower lobe resection (N=2). Lung compliance was estimated as change in lung volume divided by change in transpulmonary pressure (alveolar minus esophageal pressure). Average total lung compliance in control was 3.3±0.8 (SD) ml/cmH2O. Hydrothorax (8 ml) decreased significantly ($P<0.001$) lung compliance to 2.7±0.7 ml/cmH2O and increased pleural liquid pressure at bottom of the cavity from -1 cmH2O up to ~2.5-3 cmH2O. The remarkable decrease in compliance was attributed to the fact that with hydrothorax the lung was exposed to positive, rather than sub-atmospheric, pressure causing atelectasis. Resection of right lower lobe decreased significantly ($P<0.001$) right lung compliance to 1.75±0.3 ml/cmH2O. Resection of right middle plus lower lobes decreased significantly ($P<0.001$) lung compliance to 1.52 ±0.4 ml/cmH2O. In both cases, the decrease in compliance was greater than expected based on the reduction in lung volume. We conclude that a potential combined detrimental effects of hydrothorax and lobar resection to decrease lung compliance expose the lung to the risk of overdistension when a chest drainage is applied.

Abstracts

Poster Session 1

Topic 1: Neurobiology and Neurophysiology

Topic 2: Metabolism, Nutrition and System Physiology

Topic 3: Comparative and Environmental Physiology, Joint Workshop

P1.1

Study of the effects of nanoliposomes engineered for the treatment of Alzheimer's disease on the electrical activity of cortical neurons

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Nano-carries represent promising tool in the treatment of Alzheimer's disease (AD): they can be specifically tuned improving qualitatively and quantitatively the transport of drugs to the central nervous system, limiting side effects.
We evaluated the impact of nanoliposomes (NL), functionalized to cross the blood-brain barrier and to bind Aβ, on neonatal rats primary cortical neurons.
Biocompatibility studies done on cells incubated with 10 μM NL revealed that up to 48 hours (h), the LDH release and the mitochondrial distress were irrelevant (1±1% and 4±0.5%, respectively). Patch clamp, in voltage and current clamp mode, was conducted on mature excitatory pyramidal neurons after 4 (4h) or 24 (24h) h of NL incubations. Current threshold required to activate the action potentials firing decreased if neurons were incubated for 4h with NL being 30±2 pA in control (CT); 24±3pA in 4h ($p<0.05$) and 28±5 in 24h. The frequency of firing was 12±1Hz in CT, 16.8±2Hz in 4h ($p<0.05$) and returned to 12±2Hz in 24h. Cells capacitance was not affected, but membrane resistance tended to increase with time up to 4h of NL exposure and went back for prolonged incubation (0.7±0.07 GΩ in CT and 0.8±0.1 GΩ, 0.6±0.1 GΩ in 4 and 24h respectively). Moreover, confocal microscopy revealed that NL were not uptaken by neurons.
In conclusion, we demonstrated that biocompatible NL functionalized for AD treatment transiently influenced the electrical activity of cortical neurons, without being uptaken.

P1.2

Coding of goal-directed actions in ventrolateral prefrontal and ventral premotor cortex

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Ventrolateral prefrontal (VLPF) cortex is deemed to play a number of motor-related functions, but its contribution to the organization of goal-directed natural actions is still unknown. Here we recorded VLPF and ventral premotor (area F5) neurons of one monkey in a task in which an object or a piece of food elicited two different actions, grasp-to-place or grasp-to-eat, respectively. We analysed 342 VLPF and 222 F5 task-related neurons. Most (45%) of VLPF neurons responded to target presentation, 9% activated only during reaching-grasping, while 29% discharged during both visual presentation and movement execution. Nearly all F5 neurons discharged during motor execution. Fifty-three percent of them also activated during target presentation. Interestingly, although a visual and motor selectivity for the type of target was rare in VLPF single neurons, a clear preference for the object with respect to food emerged at the population level, both during target presentation and motor execution. In contrast, F5 single neurons showed a differential, action goal-dependent, discharge during visual presentation (33%) and during grasping execution (50%). Together with previous findings, these results suggest that goal-directed natural actions might be mainly organized by parieto-premotor circuits, by activating specific motor sequences afforded by the type of target object. In contrast, VLPF might play a role when the action has to be learned through an explicit training process.

P1.3

Beyond the cholinergic activity of NGF: rescue of Alzheimer-like neurodegeneration by painless NGF acting on APP processing and glial cells

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The clinical application of Nerve Growth Factor (NGF) to prevent or slow human neurodegenerative diseases, such as Alzheimer's disease, is limited by the adverse effects of NGF in activating nociceptive responses. We developed a human NGF double mutant (hNGFP61S/R100E), inspired by the HSAN V mutation in the NGFB gene, that has identical neurotrophic properties to human NGF, is traceable against endogenous NGF and has a greatly reduced ability to activate nociception.
The objectives of this study are to assess the therapeutic efficacy of hNGFP61S/R100E in transgenic mice harboring five familiar AD-related mutations (5xFAD mice), and to investigate the mechanisms underlying the rescue of neurodegeneration in these mice, as well as the absence or presence of nociception after intranasal delivery.
We demonstrate that hNGFP61S/R100E, delivered after the onset of neurodegeneration, induces a complete rescue of spatial memory and synaptic plasticity deficits and a decrease in the plaque load in 5xFAD mice. The mechanisms underlying these effects are linked to a clear reduction pathological APP processing and astrocytosis and an increase of microglia phagocytic activity. Moreover, we demonstrate that both acute and chronic intranasal administration of painless NGF does not trigger pain in 5XFAD mice, measured at the behavioural level.
Thus, these findings confirm that hNGFP61S/R100E is a viable option to increase NGF activity in the brain in a non invasive way, increasing its pharmacological therapeutic window and provide further proof that the neuroprotective activity of NGF goes well beyond the expected neurotrophic activity on cholinergic neurons.

P1.4

Low vagally-mediated heart rate variability and increased susceptibility to ventricular arrhythmias in rats bred for high anxiety

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In humans, there is a documented association between anxiety disorders and cardiovascular disease. Putative underlying mechanisms may include an impairment of the autonomic neural control over cardiac function. In this study we characterize cardiac autonomic modulation in genetic lines of rats that differ largely in their anxiety level. ECGs were recorded in high-anxiety behavior (HAB, n=10) and low-anxiety behavior (LAB, n=10) rats at rest, during stressful stimuli and under autonomic pharmacological manipulations, and analyzed by means of indexes of heart rate variability. In resting conditions, HAB rats displayed reduced heart rate variability, mostly in terms of lower vagal modulation compared to LABs. In HAB rats, this relative low vagal autonomic regulation was associated to a smaller heart rate responsiveness to acute stressors. In addition, beta-adrenergic pharmacological stimulation induced a larger incidence of ventricular tachyarrhythmias in HAB rats.
We conclude that high levels of anxiety-related behavior in rats are associated with signs of i) impaired cardiac autonomic modulation (low vagally-mediated heart rate variability), ii) poor adaptive heart rate responsiveness to stressful stimuli and iii) increased arrhythmogenic susceptibility, which may predict increased vulnerability to cardiac disease. These results highlight the utility of the HAB/LAB model for investigating the mechanistic basis of the comorbidity between anxiety and cardiovascular disorders.

P1.5

Lisinopril, but not losartan microinjected into the caudal nucleus tractus solitarii potentiates the cough reflex in the rabbit

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The caudal nucleus tractus solitarii (cNTS), the predominant site of termination of cough-related afferents, has been shown to be a site of action of some centrally acting antitussive agents and a component of a drug-sensitive gating mechanism of cough. We have provided evidence in both anesthetized and awake rabbits that the ACE inhibitor lisinopril, but not the angiotensin II receptor blocker losartan, increases the number of coughs induced by both mechanical and chemical stimulation of the tracheobronchial tree. Since both lisinopril and losartan cross the blood-brain barrier, a central action at the level of cough-related brainstem structures, and especially of the cNTS, can be suggested. To this purpose, bilateral microinjections (30-50 nl) of losartan (5 mM) and lisinopril (1mM) were performed into the cNTS of pentobarbital sodium-anesthetized, spontaneously breathing rabbits. The cough reflex was induced by citric acid inhalation and mechanical stimulation of the tracheobronchial tree. Lisinopril consistently increased the number of coughs induced by both mechanical and chemical stimulation. The recovery was observed within ~ 40-60 min. On the contrary, losartan at the same cNTS sites had no appreciable effects. The results support the hypothesis of a central action of lisinopril possibly via a local cascade of effects starting with the accumulation of bradykinin and involving other biochemical events such as the production of prostaglandins, NO and substance P.

P1.6

Vaccinium Myrtillus anthocyanosides long-term intake and hamster pial microcirculation during ischemia-reperfusion injury

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Vaccinium Myrtillus anthocyanosides *(VMA)* have been shown to prevent glaucoma, coronary heart disease and peripheral microvascular dysfunctions. This study was aimed to assess *in vivo* hamster pial microvascular responses to hypoperfusion-reperfusion injury (H/R) after long-term intake of VMA. Hypoperfusion was induced by bilateral common carotid artery occlusion (BCCAO) for 30 min followed by 60 min of reperfusion. The pial microcirculation was visualized by fluorescence microscopy. VMA [10 mg/100 g b. w.] were orally administered for 6 or 8 months. Arteriolar diameter, microvascular permeability increase, leukocyte adhesion, perfused capillary length (PCL) and capillary red blood cell velocity (VRBC) were evaluated. Pial arterioles were classified by Strahler's ordering scheme. BCCAO caused marked decrease in order 3 arteriole diameter that was reduced by 11.5 ± 2.0% of baseline at the end of reperfusion (RE). Microvascular permeability and leukocyte adhesion were pronounced; PCL and VRBC decreased at RE. VMA treatment preserved the pial microvasculature from damage induced by H/R, inducing arteriolar dilation by 10.5 ± 1.5% of baseline, preventing permeability increase, leukocyte adhesion, occlusion of capillaries and decrease in VRBC. These protective effects were more pronounced in hamsters treated with VMA for 8 months. In conclusion, long-term intake of VMA prevents pial microvascular damage due to H/R, preserving blood brain barrier integrity and capillary perfusion.

P1.7

Synaptosomal protein synthesis from rat brain: more than one system?

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Synaptosomal protein synthesis from rat brain is selectively increased by learning and massively enhanced during recovery from brain ischemia, thus indicating its substantial contribution to brain plastic responses. To identify the nature of the involved synaptic components, synaptosomal protein synthesis was assayed under markedly modified concentrations of extracellular cations and endogenous calcium. Most rate response curves obtained under these conditions exhibited biphasic profiles suggesting the participation of more than one translation system. Comparable results have been reproduced under conditions of full inhibition of mitochondrial protein synthesis. Our data suggest that cytoplasmic translation systems of nerve terminals, postsynaptic sites or fragmented glial processes are differentially responding to the modified concentrations of external and endogenous cations. The subsynaptic localization and main properties of such systems may now be identified by the colocalization of specific antibodies with fluorogenetic proteins synthesized under proper assay conditions.

P1.8

Resilience to audiogenic seizures is associated with p-ERK1/2 dephosphorylation in the subiculum of a mice model of Fragile X syndrome

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Fragile X syndrome (FXS) is the most common form of mental retardation. The neurologic phenotype of FXS includes epilepsy and hyper-reaction to sensory stimuli, and it is well reproduced in the *Fmr1* knockout (KO) mice. *Fmr1* KO mice display audiogenic seizures (AGS), and seizure susceptibility is age-dependent.
To characterize the response to AGS, we delivered a loud acoustic stimulus (~122 dB) in *Fmr1* KO and wild type (WT) mice at postnatal day (P) 45 and P90. The activity markers FosB/ΔFosB and phosphorylated-extracellular signal-regulated kinases 1/2 (p-ERK1/2) were evaluated by immunohistochemistry.
WT mice did not show AGS, while *Fmr1* KO mice displayed AGS with age-dependency (100% at P45, 25% at P90). FosB/ΔFosB immunoreactivity increased in medial geniculate body and CA3 at P45, but not at P90, after acoustic test in WT and *Fmr1* KO mice. p-ERK1/2-immunopositive neurons were more abundant in the subiculum of *Fmr1* KO mice at P45 and P90 compared to WT, and increased, after the acoustic test, in P45 and P90 WT mice, while decreased in P90 and did not change in P45 *Fmr1* KO mice.
Our findings illustrate that: (i) FosB/ΔFosB markers are overexpressed in the medial geniculate body and CA3 in *Fmr1* KO mice experiencing AGS; (ii) p-ERK1/2 is markedly decreased in the subiculum of *Fmr1* KO mice resistant to AGS induction. These data suggest that resilience to AGS is associated with dephosphorylation of p-ERK1/2 in the subiculum of mature *Fmr1* KO mice.

P1.9

Early axonal growth of hippocampal neurons is reduced and less sensitive to BDNF in dystrophic *mdx* mice compared to wild-type

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Dystrophin (Dp427) is a cytoskeletal protein defective in muscle and brain of Duchenne muscular dystrophy patients and *mdx* mice. Differently from muscle, neuronal damages caused by lack of Dp427 are established during development and are not progressive, suggesting different functions of this protein in pre and post-natal stages. We previously reported a role for dystrophin in NGF-dependent axonal growth and regeneration in sympathetic neurons, both *in vivo* and *in vitro*. Aim of the present study was to analyze whether Dp427 may also be important for central hippocampal neurons development, in the presence or not of the differentiating brain derived neurotrophic factor (BDNF). We cultivated hippocampal neurons for 6 days (6DIV) in microfluidic chambers, which allow specific axonal growth into a compartment separated from dendrites and cell bodies. As *in vivo*, WT hippocampal neurons *in vitro* express Dp427 and proteins of the complex to which it associates. By 3DIV, the number of axons crossing the channels separating the two compartments was lower in *mdx* mouse neuron cultures respect to WT. This difference was significantly higher when 50 ng/ml BDNF were added to the medium. Our data indicate that, although BDNF is not important for hippocampal neuron survival, it enhances early axonal growth and that this effect is milder in *mdx* mouse neurons respect to WT. This further corroborates the hypothesis that Dp427 plays a role in neurotrophin-dependent axonal development.

P1.10

Neural activity associated to joint-action during social cooperation in frontal and parietal cortex of macaque monkeys

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The neural mechanisms related to the ability of non-human primates to interact in a social context are still unknown. So far, neural activity associated to cognitive-motor functions has been studied in a single brain, thus missing all information typical of interacting brains. We recorded extracellular single-unit activity simultaneously from homologous areas [premotor cortex (PMd) and inferior parietal cortex (IPL)] of two monkeys, while they cooperated in a joint-action task. The two animals were trained to move a cursor through an isometric joystick in two conditions: a "SELF" condition, where each monkey moved individually its cursor toward peripheral targets; a cooperation condition ("COOP"), where both monkeys moved their cursors in a coordinate fashion toward the same target, with spatio-temporal constraints. We recorded the activity from 540 cells in PMd and 258 in IPL. The activity of 28.5 % cells in PMd and 22.1% in IPL was significantly different in the SELF and COOP trials, despite the similarity in kinematics observed across tasks. Significant changes were also observed in directional properties of 13.7% PMd cells and 14.3% IPL neurons. Therefore joint-action influences both the firing rate and directional tuning of parietal and frontal neurons, both in terms of gain and shift of preferred direction, relative to individual action. These results represent a first step toward understanding the neural operations underlying motor functions in a cooperative context.

P1.11

Neural stem cells-enriched tubulization improves anatomical and functional restoration of the severed rat sciatic nerve

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Reconstruction of the severed peripheral nerve entails direct suturing or, in case of extensive gap, tubulization of the nerve using adequate biocompatible conduits. Loading tubules with growth-promoting factors or cells may speed-up and/or optimize nerve repair, however, only relatively scant evidence is available. Here, this possibility has been investigated after the formation of a 5-mm gap and tubulization of the rat sciatic nerve with a cell-compatible, biodegradable PLCL (poly DL-lactide-e-caprolactone, Neurolac®) copolyestere tube filled with either cultured Human Umbilical Mesenchymal Stem Cells (HUMSCs), human neural progenitors or their conditioned medium. The study included also animals subjected to direct suturing of the transected nerve, animals with gap only and animals with gap and unloaded PLCL tube. Starting from one week up to 5 months post-surgery, behavioural tests were administered weekly, followed by histochemical and tract-tracing analyses to assess the anatomical and functional condition of the treated nerve, with respect to the intact contralateral side. The results showed a remarkable nerve integrity and a better functional recovery in the animals implanted with the cell- or medium-loaded tube, compared to the other groups. Thus, tubulization associated with local supply of growth-promoting factors may represent a viable strategy for optimizing functional restoration of the peripheral nerve, but further analyses are needed before its clinical use.

P1.12

On the role of K^+ channels in autism spectrum disorders

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Autism spectrum disorders (ASD) are characterized by impaired ability to properly implement environmental stimuli that are essential to achieve a state of cultural and social inter-relationships. The main features of this disease are marked impairments of verbal and non verbal communication with restricted and repetitive behaviors and epilepsy. Mutations in genes whose products are crucial for the development and plasticity of such neuronal circuits may drive the network to stalemate and lead to irreversible consequences. Here we report on the direct and indirect involvement of K^+ channels in a number of neurological and psychiatric disorders including ASD, epilepsy, and mental retardation. Genomic scan analysis of ASD/epilepsy patients showed deleterious mutations in the inwardly-rectifying K^+ channel. Kir channels are widely expressed in astrocytes and oligodendrocytes where this channel type plays an active role in K^+ siphoning, and modulates neurotransmitters uptake and release from astrocytes, affecting tripartite synapse functionality, long distance neuronal firing, and brain wiring. Since K^+ channels play central roles in cortical development and connectivity, defects in their genes may lead to cognitive and behavioral impairments typical of ASD. Thus, this study focuses on the role of K^+ channels in ASD and analyzes the clinical data, genetic findings, and functional properties of several mutations identified in these channels.

P1.13

The human olfactory threshold: new physiological insight on its senescence

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The sense of smell is evolutionary settled on a physiological multidimensional space; such complexity allowed the system to generate electrical signals processed and coded in the olfactory bulb and distributed to several areas in the brain. This mechanism is under great scrutiny due to the complexity of understanding how an enormous number of chemically diverse molecules are coded into signals detected by the different brain areas. Additionally, it has been challenging to dissect olfactory perception due to the multiple areas of the brain that receive and modulate this information. Consequently, our knowledge of olfactory function in humans remains primitive. Aging represents the major cause of loss of smell; to investigate it we disassembled olfactory function in its principal physiological component, the threshold. In healthy population sample (N =600, AGE = 29.3±15.3, range 5-86) we established the age olfactory threshold database. The threshold is: whole population 4.24±1.7; male 4.28±1.6; female 4.23±1.7 (ovulatory 4.27±1.6; no-ovulatory 3.9±1.5; oral contraceptive taker 4.65±1.6 SD; menopause 5.9±1.8 SD). Interestingly male and female threshold are only significantly different in the age clusters 40 and 50 years. Furthermore, we found three phenotype of threshold that putatively can be termed young, mature and senescent. The present study provides new physiological insight on variability and senescence of the olfactory threshold in healthy population.

P1.14

Alpha-tocopherol reduces neuroinflammation in the rat brain after kainic acid-induced status epilepticus

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α-tocopherol (α-T) has beneficial effects in epilepsy, mainly ascribed to its antioxidant properties. Besides radical-induced neurotoxicity, neuroinflammation is also involved in the pathophysiology of epilepsy. We have previously shown that feeding rats for two weeks with α-T-enriched diet before inducing status epilepticus reduces astrocytic and microglial activation, neuronal death and oxidative stress in the hippocampus.
Here, we assessed whether similar anti-inflammatory effects could be observed when α-T supplementation was administered post-seizure induction. Seizures were induced in adult male rats by kainic acid injection; 3 hours after status epilepticus, some rats received an intraperitoneal bolus of 250 mg/kg α-T (once a day, for 4 days), whereas control rats were injected with vehicle. At the end of treatment, hippocampi were processed for immunohistochemistry and western blotting.
α-T promoted marked anti-inflammatory effects, that could be summarized as follows: reduced astrocytic GFAP-immunoreactivity, and enhanced expression of glutamine-synthase; increased length and complexity of Iba-1-positive microglial processes; reduction of pro-inflammatory cytokine expression (IL-1β and TNF-α).
These results clearly indicate that a brief period of supplementation with α-T, initiated immediately after seizure, potently reduces neuroglial activation occurring after status epilepticus, thus emphasizing anti-inflammatory role of α-T in epilepsy.

P1.15

Local field potentials are influenced by cooperative joint-action in frontal and parietal cortex of macaque monkeys

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We studied local field potentials (LFPs:1-100 Hz) from dorsal premotor (PMd) and inferior parietal cortex (IPL) in two monkeys, to determine the context influence on neural activity of an obligatory joint-action with a partner.
Monkeys were trained in a center-out task in two conditions. In the first (SELF) each animal moved its cursor from the center toward a peripheral target, by applying a force pulse on an isometric joystick. In the second (COOP), both monkeys moved their cursors simultaneously toward the same target, constrained by a maximum inter-cursor distance.
We recorded neural activity from 236 PMd and 166 IPL sites, simultaneously from homologous areas of both monkeys and defined two epochs of interest, reaction time (RT: 0.2 s from target presentation) and movement time (MT: 0.2 s from movement onset). The peak-to-peak LFP amplitude in each epoch was compared between the SELF and COOP conditions and across target directions.
A two way ANOVA showed a significant difference of LFP amplitude during RT or MT between SELF and COOP conditions in 25.8 % PMd and 21.1% IPL sites, and between target directions in 41.9% PMd and 36.7 % IPL sites, with a significant interaction factor in 11% PM and 13% IPL sites.
We conclude that joint action modulates both the amplitude and the directional properties of LFPs associated to individual action, suggesting that there exists in the parieto-frontal system an action cooperation network set in motion during joint movement.

P1.16

Expression of Wilms' Tumor protein (WT1) in developing human peripheral sympathetic and gastroenteric nervous system

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Developmental expression of Wilms' tumor gene (WT1) and protein is crucial for cell proliferation, apoptosis, differentiation and cytoskeletal architecture regulation. Recently, a potential role of WT1 has been suggested in the development of neural tissue and in neurodegenerative disorders. Accordingly, we have investigated immunohistochemically the developmentally regulated expression and distribution of WT1 in the human fetal peripheral sympathetic nervous system (PSNS) and the gastro-enteric nervous system (GENS) from weeks 8 to 28 gestational age. WT1 expression was restricted to the cytoplasm of sympathetic neuroblasts, while it progressively disappeared with advancing morphologic differentiation of these cells along both ganglionic and chromaffin cell lineages. In adult tissues, both ganglion and chromaffin cells lacked any WT1 expression. The transient WT1 expression in sympathetic neuroblasts, associated with progressive loss in ganglion and chromaffin cells, suggests its potential repressor role of differentiation in a precise temporal window during the development of the human PSNS and GENS. The cytoplasmic localization in human fetal neuroblasts, as previously described in skeletal/cardiac muscle cells and in endothelial cells, is in line with WT1 involvement not only in nuclear transcriptional regulation, but also in cytoplasmic RNA metabolism and translational regulation by acting through nucleocytoplasmic shuttling properties.

P1.17

Post-natal developmental alterations in the retina of dystrophic mdx mice

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Duchenne muscular dystrophy (DMD) is an X-linked myodegenerative disease caused by defective expression of full-length dystrophin (Dp427) consequent to mutations in the DMD gene. Besides muscle degeneration, DMD patients also experience forms of cognitive impairment, altered scotopic electroretinogram and red-green color vision defects. Here, we evaluated differences in both morphological differentiation of the retinal layers and gene expression during development of wild type (WT) and dystrophic mdx mouse retina. Differentiation of retinal layers, a phenomenon occurring after birth, was analyzed on paraffin-embedded eye sections, from P5, P10 and 6-7 weeks old mice. Thickness of layers was measured and statistically compared. A significant reduction in the thickness of both the outer segment of photoreceptors (OSP) and ganglion cell layer (GCL) was observed at P5, when retinal cells are still migrating in their final position. Moreover, at P5, segregation of the different cells in the appropriate layer starts to become evident only in WT mouse retina, suggesting an early asynchronous migration in mdx mice. All these gross dissimilarities disappear in older mice. Differences in mRNA expression between the two genotypes was evaluated by RNA Sequencing on P5 retinas. About 50 genes were significantly up- or down-regulated in mdx mice compared to WT. The majority of the down-regulated genes was related to cell development, transcription, cytoskeleton dynamics and cell stress response. In conclusion, our data suggest that, as in other nervous system regions, also in the retina Dp427 plays a major role in neural development and early differentiation.

P1.18

Identification of anti-neuronal antibodies in the serum of patients with Tourette's syndrome

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Tourette syndrome (TS) is a chronic multiple-tic disorder affecting 0.8 to 1% of the pediatric population. Consistent data support the presence of abnormalities of immune regulation in children with TS. Environmental factors acting on the immune system, such as group A Streptococcus (GAS) infection, could modify the course of the severity of tics and symptoms in TS.
Structural similarity between streptococcal and cerebral antigens might elicit a pathogenic cross-reactivity of antibodies originally targeting GAS antigens to host antigens. Molecular mimicry models has been proposed involving cross-reactivity between streptococcal and neuronal proteins. Given the role of dopamine and dopamine receptors in the control of movement and behavior, we hypothesized that patients with TS harbored serum autoantibodies against dopamine receptors.
In our work, we screen and measure the anti-neuronal antibodies in TS patient's sera. Specifically, we use flow cytometry cell-based assay to test sera for the presence of autoantibodies binding to dopamine D2 (DRD2) and D1 receptors (DRD1) on surface of transfected HEK293 cells.
Within the European Multicentre Tics in Children Studies project, financed under the 7th Framework Programme of the European Union, we measure IgG reactivity to DRD2, DRD1, of a thousand of Tourette's sera from all Europe. The data thus obtained will present high statistical power and will represent the starting point for the diagnostic methodology of TS.

P1.19

Identification of a point mutation impairing the binding between Aquaporin-4 and the Neuromyelitis Optica autoantibodies

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Neuromyelitis Optica (NMO) is characterized by the presence of autoantibodies (NMO-IgG) against Aquaporin-4 (AQP4) supra-molecular assemblies, called Orthogonal Array of Particles (OAPs). The characterization of the interactions behind the formation of epitopes is essential to provide an effective NMO treatment and to elucidate pathogenesis. The aim of this study was the identification of aminoacid important for OAP formation and NMO-IgG binding. Alignment analysis of AQP4, showed that the trans-membrane regions (TMs) are highly conserved due to a number of structural-functional constraints. By mutagenesis of TMs and AQP4 chimeras, we have highlighted the key role of Aspartate69 (D69) in TM2 assembly of NMO-IgG epitopes. It was in fact observed that the mutation from D69 to histidine reduced approximately by 10 fold the binding of NMO-IgG to AQP4. Although BN-PAGE and TIRFM indicated that OAPs were similar, molecular dynamics simulations revealed that D69 mutation had the effect of inducing structural rearrangements of the extracellular loop A and thus affecting the stability of the OAPs assembly. In conclusion, D69 is crucial for the spatial position of loopA and for the correct assembly of OAPs and NMO-IgG epitopes. These findings may provide additional clues for envisaging new strategies for NMO treatment and new information about NMO pathogenesis.

P1.20

F3/contactin promotes hippocampal neurogenesis, synaptic plasticity and memory in adult mice

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F3/contactin, a cell-adhesion molecule belonging to the immunoglobulin supergene family, is involved in several aspects of neural development including synapse building, maintenance and functioning. Here we examine F3/contactin function in adult hippocampal neurogenesis, synaptic plasticity and in memory, using as a model TAG/F3 transgenic mice, where F3/contactin overexpression was induced under control of regulatory sequences from the human TAG-1 (TAX-1) gene. Transgenic mice aged 5 (M5) and 12 (M12) months exhibited an increase in hippocampal size, which correlated with positive effects on precursor proliferation and NeuN expression, these data suggesting a possible role for F3/contactin in promoting late hippocampal development. On the functional level, TAG/F3 mice exhibited increased CA1 long-term potentiation and improved spatial reference and object recognition memory, notably at 12 months of age. Interestingly, these mice showed an increased expression of the phosphorylated transcription factor and memory molecule CREB, which may represent the main molecular correlate of the observed developmental and functional effects. Altogether, these findings indicate for the first time that F3/contactin plays a role in adult hippocampal neurogenesis and that this effect correlates with improved synaptic function and memory.

P1.21

Characterization of cognitive deficits in rats with selective cholinergic, noradrenergic and dopaminergic lesions

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Besides classic motor symptoms associated to the loss of nigro-striatal neurons, cognitive deficits and dementia are now emerging as important non-motor features of Parkinson's disease (PD). Noradrenergic (NA), cholinergic (ACh) and dopaminergic (DA) neurons in the Locus Coeruleus (LC), Basal Forebrain (BF) and Ventral Tegmental Area (VTA), respectively, degenerate early in PD and appear to be involved in its non-motor manifestations, however their role has so far been much less studied. Here, we sought to address this issue in the rat, by producing selective immuno- and neurotoxic lesions, either single or combined, in order to investigate the occurrence of possible interactions between transmitter systems in the production of cognitive deficits. Starting from 12 weeks post-surgery, the animals were tested in the *Morris Water Maze* (MWM) and the *Radial Arm Water Maze* (RAWM) tasks, specifically designed to evaluate reference and working memory abilities. All animals with single lesions did not show significant impairments in the reference memory task compared to control. By contrast, significant working memory deficits were exhibited by the single-lesioned animals, being seen more pronounced in the double- and particularly severe in triple-lesioned animals. The results suggest that monoaminergic neuron systems may functionally interact for sustaining normal cognitive abilities, their dysfunction being possibly responsible for several of the non-motor symptoms of PD.

P1.22

Novel domain architecture for sacsin protein using comparative analysis and functional mapping of human *SACS* mutations

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disease due to mutations of the *SACS* gene, which encodes sacsin, a multidomain protein of 4579 amino acids with chaperone-like activity. The enormous size of *SACS* gene and translated protein has hindered biochemical analysis of ARSACS, and how mutant sacsins lead to neurodegeneration is largely unknown. A variety of domains have been recognized along sacsin, included three repeated domains (~360 residues), called Sacsin Repeated Regions (SRR), that seem to contribute to its chaperone-like activity. In this study, we found that the three repeated regions are much larger (≥1100 amino acids) than those previously described and are organized in sub-repeats. We named the large repeated regions *Sacsin Internal RePeaTs* (*SIRPT1*, *SIRPT2* and *SIRPT3*) and the sub-repeats *sr1*, *sr2*, *sr3* and *srX*. Such organization in domains was conserved in vertebrates. Comparative analysis of vertebrate sacsins in combination with fine positional mapping of a set of human mutations revealed that *sr1*, *sr2*, *sr3* and *srX* are functional. Furthermore, correlation between location of the pathogenic mutations and severity of the clinical phenotype (assessed by a severity scoring system) suggests different functional relevance of the four sub-repeats in sacsin activity. In perspective, the definition of the role of such domains may help in developing a model of function for sacsin in the context of cell pathophysiology.

P1.23

Development of motor cooperation through joint-action

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The development of joint-action was studied in 37 age and gender-matched couples of children (5- 9 years old), and in 12 couples of adults (27±7 ys old). Subjects acted on an isometric joystick, as to move a cursor toward a target under three conditions: 1) SELF: subjects acted individually; 2) JOINT-ACTION (JA): subjects guided their cursors jointly to the common target; 3) SIM: each subject coordinated his cursor's trajectory with that of the partner, however simulated by the computer. In JA and SIM, the between cursors distance had to remain within a given threshold. In SELF, the success rate (SR) increased linearly with age, reaching the adult performance at 8 ys. A similar trend was observed in JA and SIM. At 8 ys, the SR during JA was significantly higher than that of younger children. However, in JA and SIM maturity was only reached at 9 ys. Despite the increase of performance through ages in both JA and SIM, only at 8y the SR in JA became higher than that of the SIM, showing that the presence of an interacting partner improves performance. This *cooperation benefit* reversed an opposite trend in younger kids. The improvement of JA with age was correlated with a slowdown of the MTs, and a linear decrease of the between cursors distance. The maturation of joint-action emerges as a progressive shaping of individual movement parameters and their adaptation to those of the partner, with a benefit emerging from an effective, as opposed to a simulated cooperation.

P1.24

Vagal withdrawal and cardiac arrhythmia vulnerability in rats with high trait aggressiveness

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Personality characteristics, e.g. aggressiveness, have long been associated with an increased risk of cardiac disease. However, the underlying mechanisms remain unclear. In this study we characterized cardiac autonomic modulation in rats that differ widely in their level of aggressive behavior. High-aggressive (HA, n=10) and non-aggressive (NA, n=10) rats were selected from a population (n=121) of adult male wild-type rats on the basis of their latency time to attack (ALT, s) a male intruder in a resident-intruder test. ECG recordings were obtained via radiotelemetry at rest, during stressful stimuli and under autonomic pharmacological challenge, and analyzed via time- and frequency-domain indexes of heart rate variability. In resting conditions, HA rats (ALT<90s) displayed reduced vagal modulation compared to NA rats (ALT>600s). Exposure to stressful stimuli (restraint and psychosocial stress) provoked similar tachycardic responses in the two groups. However, under stress conditions HA rats displayed a reduced vagal antagonism and an increased incidence of tachyarrhythmias compared to NA rats. In addition, beta-adrenergic pharmacological stimulation induced a much larger incidence of ventricular tachyarrhythmias in HA rats. These findings are consistent with the view that high levels of aggressiveness in rats are associated to signs of cardiac autonomic impairment and increased susceptibility to arrhythmias, that may predict vulnerability to cardiac morbidity and mortality.

P1.25

Multiple effects of selective cholinergic lesions combined with local infusion of pre-aggregated amyloid peptide

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Cholinergic loss, amyloid peptide deposition and tau hyperphosphorylation are important hallmarks of Alzheimer's Disease (AD). Besides, overexpression and aggregation of transactive response DNA- binding protein 43 (TDP-43) have been associated to the disease. However, it is not known whether these features interact in AD. Here, the possible relationships between the various hallmarks in producing cognitive deficits have been addressed by combining selective lesioning of basal forebrain cholinergic neurons with hippocampal injection of pre-aggregated beta (25-35) amyloid peptide, the latter giving rise to local accumulation of amyloid oligomers and protofibrils. Four to five weeks post-surgery, the animals were subjected to sequential behavioural tasks aimed at evaluating reference and working memory abilities, followed by post-mortem histo- and immunohistochemistry, as well as western blot and RT-PCR assessments. The results show robust deficits in both reference and working memory, associated to widespread cholinergic depletions, the occurrence of amyloid aggregates in the neocortex and hippocampus, marked regional increases of APP and tau levels, as well as abnormal TDP-43 mRNA expression levels, which were seen more pronounced in the animals subjected to double, but not to either single treatment. Thus, amyloid, tau and TDP-43 pathologies may require association with disturbances in monoaminergic (e.g. cholinergic) neurotransmission for inducing cognitive impairments.

P1.26

Anti-aggregating effect of the naturally occurring dipeptide carnosine on Aβ1-42 fibril formation

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Carnosine is an endogenous dipeptide abundant in the central nervous system, where by acting as intracellular pH buffering molecule, Zn/Cu ion chelator, antioxidant and anti-crosslinking agent, it exerts a well-recognized multi-protective homeostatic function for neuronal and non-neuronal cells. Carnosine seems to counteract proteotoxicity and protein accumulation in neurodegenerative conditions, such as Alzheimer's Disease (AD). However, its direct impact on the dynamics of AD-related fibril formation remains uninvestigated. We considered the effects of carnosine on the formation of fibrils/aggregates of the amyloidogenic peptide fragment Aβ1-42, a major hallmark of AD injury. Atomic force microscopy and thioflavin T assays showed inhibition of Aβ1-42 fibrillogenesis *in vitro* and differences in the aggregation state of Aβ1-42 small pre-fibrillar structures (monomers and small oligomers) in the presence of carnosine. Inhibition of formation of amyloid aggregates by carnosine was shown to be dose-dependent in the 0-10 mM concentration range. *In silico* molecular docking supported the experimental data, calculating possible conformational carnosine/Aβ1-42 interactions. Overall, our results suggest an effective role of carnosine against Aβ1-42 aggregation.

P1.27

A visuomotor disorder in absence of movement: optic ataxia generalizes to learned isometric hand action

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Parietal cortex (PPC) lesion results in Optic Ataxia (OA), a disorder of reaching to visual targets. It is unknown whether such lesion results in similar defect under isometric conditions, in absence of a physical displacement of the arm, therefore regardless of the the sensor-motor transformation required. We asked a patient with OA from a unilateral tumor lesion of the right superior parietal lobule (SPL) and a group of control subjects to perform a center-out task involving either natural reaching movements toward peripheral targets, or a learned isometric hand action as to move a visual cursor toward the same targets. Both tasks were performed with or without fixation of a central light, as to present reach targets in peripheral or central vision. Learned isometric action was affected similarly to natural reaches in both central and peripheral vision, with abnormal endpoint errors and spatial dispersion of cursor trajectories. Perceptual and motor aspects of hand errors were dissociated showing that OA consists of both spatial and motor components, since a field effect emerged in the process of target localization, in addition to a hand effect observed only for the motor components of OA. This suggests that lesion of PPC affects sensory-motor transformations not only during natural hand movements, but also when visual signals about target location need to be aligned with information from hand force receptors, therefore regardless of the specific remapping required.

P1.28

Progressive motoneuronal degeneration and motor dysfunction in sod1g93a mice: effects of implanted mesenchymal stem cells from human umbilical cord (HUMSCS)

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Transplantation of Mesenchymal Stem Cells from the Human Umbilical Cord (HUMSCs) has recently emerged as a promising strategy for the treatment of disorders such as Amyotrophic Lateral Sclerosis (ALS), a disease characterized by progressive loss of motor neurons in cortex, brainstem, and spinal cord. However, extensive pre-clinical characterization of these cells is necessary, prior to their clinical use. Here, the functional properties of HUMSCs were investigated following implantation in SOD1G93A mice, a well-known ALS model. HUMSCs, cultured and primed using a neuron-conditioned medium, were implanted into the lateral ventricle of newborn (post-natal day 4) SOD1G93A mice. The distribution of grafted HUMSCs and their effects on disease onset and progression, motor performance, and motoneuron numbers and morphology, were assessed relative to control sham- or non-transplanted and wild-type mice.
HUMSCs transplantation significantly delayed the appearance of the severe functional impairments typically exhibited by these animals at 4-6 months of age and extended their lifespan by about 30-40%, as opposed to non-transplanted mice. Grafted cells were found in the walls of the lateral ventricles and central canal but not in the brain or the spinal parenchyma, suggesting that production and release of locally acting factors with protective and/or antiinflammatory properties, rather than replacement of degenerating neurons, are likely responsible for the observed restorative effects.

P2.1

Plasma Bisphenol-A concentration and body fat distribution: a pilot study in an urban area

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Bisphenol-A (BPA) is a potential endocrine disruptor impacting metabolic processes suspected to increase the risk of obesity, metabolic syndrome and diabetes. The only data available on BPA exposure in Italy were collected in a rural area. Our aim was to determine BPA exposure in an urban area in northern Italy and to evaluate the association between plasma BPA level and nutritional status, body fat and its distribution. We examined 31 subjects (23 females), aged 20-47 years, with different degrees of body mass index (BMI). Total plasma fasting BPA concentration was measured by ELISA; BMI, waist circumference (WC) and body fat (BF) by anthropoplicometry. The sample presented mean age of 32,3±6,5 years, mean BMI of 25,9±7,1 kg/m^2 and mean of WC of 87,6±18,0 cm. Males were younger than females (27,9±5,1 vs 33,9±6,3 years, $p<0,05$, respectively).
Plasma BPA was measurable in all subjects with a mean concentration of 0,35±0,16 ng/ml (0,16-0,59 ng/ml, 10th and 90th percentile) without significant differences between genders. Total plasma BPA correlated with BMI ($r=0,358$, $p=0,048$) and WC ($r=0,488$, $p=0,005$). In linear regression model adjusted for sex and age, using BMI and WC as independent variables, only WC was associated with BPA plasma level ($\beta=1,133$, and 95%CI 0,001-0,025, $p=0,038$). In conclusion, the whole sample resulted exposed to BPA. Moreover, the results of this pilot study suggest that BPA plasma level is associated to central obesity.

P2.2

Ketogenic diet: a model to evaluate the effect of high fat diet on regional adiposity and glucose metabolism in humans

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Ketogenic Diet (KD) is an isocaloric high-fat (80-90%), low carbohydrate (2-5%) diet, inducing ketone bodies production, applied effectively for treatment of refractory childhood epilepsy and other neurometabolic diseases. We considered KD an unique model to study in humans the effect of high fat diet on regional adiposity and glucose metabolism and to evaluate the "overflow hypothesis" proposed by Bergman et al (Obesity. 2006;14:16S-19S) about the development of visceral adiposity, hyperinsulinemia, and insulin resistance after isocaloric high fat diet in the dog model. Body composition by anthropometry, subcutaneous (SAT) and visceral abdominal fat (VAT) by ultrasonography, glucose and lipid metabolism were evaluated before and after 12 weeks of KD in 7 children (mean age: 8,4±2,0).
After KD, BMI z-scores, VAT, SAT and total body fat were unchanged whereas fasting glucose (84,3±8,8 vs 75,7±6,1 $p<0,01$), insulin (5,5±2,8 vs 2,6±1,6 $p<0,01$), and HOMA, insulin resistance index, (1,1±0,6 vs 0,5±0,3 $p<0,01$) were significantly reduced. Lipid metabolism was unchanged. Thus, increasing fat in the diet without achieving an hypercaloric intake did not increase visceral and subcutaneous abdominal fat and did not cause peripheral insulin resistance in the short term in children. Longitudinal studies are need to provide a conclusive answer on the adaptive metabolic changes on regional adiposity and insulin resistance occurring in humans during isocaloric high fat diet.

P2.3

Antiproliferative and anti-inflammatory activities of the essential oil from fruits of *Xylopia parviflora* (A. Rich.) Benth. (Annonaceae) used in Cameroon as a culinary spice

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Epidemiological and preclinical evidence points to culinary herbs and spices as minor dietary constituents with multiple anticancer and anti-inflammatory properties. The fruits of *Xylopia parviflora* (A. Rich.) Benth. (Annonaceae) are used as spice in *Nkui* and *Nah poh*, two traditional soups of the western region of Cameroon. The aim of our work was to evaluate the antiproliferative and anti-inflammatory activity of the essential oil from fruits of *X. parviflora*. Since the plant is used as a food we investigated the antiproliferative activity of the essential oil on HCT116 human colon carcinoma cell line, using MTT assay. The essential oil showed a high significant antiproliferative activity with IC_{50} value of 6.63 ± 0.3 μg/ml. Morphological changes, DNA fragmentation, fluorimetric analysis using propidium iodide and acridine orange, and caspase activation were investigated to understand the mechanism of action. The results showed that essential oil inhibited the growth of HCT116 cell line by inducing programmed cell death. Anti-inflammatory activity of essential oil was determined by measuring its inhibitory effect upon NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Data showed that the essential oil displayed significant NO-scavenging ability. This study indicates that essential oil of *X.parviflora* may potentially be used as dietary source of phytochemicals having beneficial effects on health.

P2.4

3,5-L-diiodothyronine (T_2) modifies the fatty acid composition of lipid droplets in an *in vitro* model of hepatosteatosis

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Free fatty acids (FFAs) are the main energy stores of the cells. In the hepatocyte, excess circulating FFAs are esterified to triacylglycerols (TAGs) and stored in cytosolic lipid droplets (LDs). Rat hepatoma FaO cells exposed to an oleate/palmitate mixture are a validated *in vitro* model of hepatosteatosis, previously used to verify the direct anti-steatotic effect of 3,5-L-diiodothyronine (T_2). This study demonstrates that T_2, besides reducing number and average size of LDs, also modifies the acyl composition of LDs by decreasing their content of saturated (SFA) *vs* monounsaturated (MUFA) fatty acids, this leading to an inversion of the SFA/MUFA ratio. The mRNA expression of the LD-associated proteins, adipose differentiation-related protein (ADRP), oxidative tissue-enriched PAT protein (OXPAT) and adipose triglyceride lipase (ATGL) was increased in 'steatotic' FaO cells, and T_2 treatment resulted in a further up-regulation suggesting mobilization of TAGs stored in LDs toward oxidative pathways. The stimulation of lipid oxidation by T_2 was confirmed by the increased expression of two rate-limiting enzymes of mitochondrial FFA oxidation, carnitine palmitoyl transferase (CPT1) and uncoupling protein 2 (UCP2). In conclusion, our data indicate that T_2 may stimulate oxidative metabolism of FFAs, in particular of SFAs, and thus may enrich of MUFAs the LDs. This action may protect the hepatic cell from an excess accumulation of the highly toxic SFAs.

P2.5

Antioxidant effect of a purified polyphenolic extract from grape skin on rat colon epithelium

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It is known the involvement of oxidative stress condition in the pathogenesis of several chronic diseases. In recent years naturally occurring phenolic compounds are attracting a great interest for their antioxidant properties.
The aim of the present study was to analyze the intracellular antioxidant effect of a grape skin polyphenolic extract on rat colon. The extract was obtained from a local cultivar of primitivo, in two different times of maturation of the grapes. The in vitro antioxidant capacity of the extract was preliminary measured by the ORAC (Oxygen Radical Absorbance Capacity) assay. Then, its intracellular antioxidant activity was analyzed in superficial colonocytes as inhibition of ROS intracellular increase induced by the exposure of colon explants to the prooxidant agent H_2O_2. Intracellular ROS (Reactive Oxygen Species) visualization was performed by confocal microscopy using the ROS sensitive probe CM-H2DCFDA.
The exposure of rat colon explant to the polyphenolic extracts for 1h produced a significant reduction of H_2O_2 induced ROS increase. The intracellular antioxidant activity of the extract was also dependent on the maturation state of the grapes.
In conclusion the results demonstrate that the exposure of rat colon to a red grape skin polyphenolic extract increases the cytosolic antioxidant capacity of colonocytes, revealing a protective role of red grape skin polyphenols on the gastrointestinal tract against the oxidative stress induced by pro-oxidant compounds.

P2.6

Metabolic adaptation induced by triiodotironine: a role for Uncoupling protein-3 (UCP3)

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Triiodothyronine (T3) affects calorigenesis and lipid metabolism, however the molecular mechanisms underlying its effects are not fully elucidated. We suggested that UCP3 is a molecular determinant for the regulation of resting metabolic rate (RMR) by T3; however a debate on the real function of UCP3 exists, and the use of mice lacking UCP3 (KO) will help to clarify this aspect.
To this aim we used wild-type (WT) and KO mice maintained at thermoneutrality and we detected: skeletal muscle mitochondrial thermogenesis and fatty acid oxidation rate, mice RMR and Energy Expenditure (EE), as well as Respiratory Quotient (RQ).
To evaluate the involvement of UCP3 in the metabolic adaptation induced by T3, we injected a single dose of the hormone (25 μg/100g bw) into WT and KO hypothyroid mice, and we measured their RMR, EE and QR.
When compared to WT, KO mice showed significantly lower RMR and EE, and slight higher RQ. An inhibition of mitochondrial thermogenesis and fatty acid oxidation rate were also observed.
T3 administration to hypothyroid mice increased their RMR and EE, it also reduced RQ, the effects being more pronounced and prolonged in WT mice. Within 48h, the increases in EE were +20% and +7% in WT and KO mice, respectively, while a significant decrease in RQ (-6%) was observed exclusively in WT mice.
These data suggest that UCP3 influences metabolic rate and lipid metabolism, moreover it plays a role in the metabolic adaptations induced by T3 in hypothyroid mice.

P2.7

Physiological impact associated with chronic simultaneous exposure to high-fat diet and persistent organic pollutant *p,p´*-diphenyldichloroethene (DDE): effects on hepatic mitochondrial functions

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The physiological impact associated with chronic simultaneous exposure to both high-fat diet and low doses of persistent organic pollutants (POPs), like p,p´-diphenyldichloroethene (DDE), is poorly understood. Given that mitochondria are one of the primary targets for toxic injury and high-fat feeding has been associated to mitochondrial dysfunction, we investigated the effect of chronic simultaneous exposure to low dose of DDE and high-fat feeding on hepatic mitochondrial functions in rats.
In preliminary experiments, we tested the effects of DDE *in vitro* on hepatic isolated mitochondria and we found that DDE (157 nmol/mg of protein) decreased oxygen consumption rates using succinate (FADH2- dependent pathways), glutamate (NADH-dependent pathway) or fatty acids as substrates as well as impaired mitochondrial integrity and energy coupling. Then, we performed the *in vivo* experiment by daily administrating DDE (10 mg/kg b.w. by gavage, corresponding to the Admissible Daily Dose) to Wistar rats simultaneously exposed to standard (10% fat J/J) or high-fat (45% fat J/J) diet for 4 weeks. The results showed that the physiological responses to chronic low dose of DDE with simultaneous exposure to standard or high-fat diet, mainly included a decrease in the degree of oxidative phosphorylation coupling probably to counteract oxidative stress and an increase in fatty acid oxidation to cope with increased energetic demand associated with hepatic detoxification mechanism.

P2.8

Effects of genistein, a soy-derived isoflavone, on endothelial function in postmenopausal women with metabolic syndrome

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Background. Previous data have suggested that soy isoflavone genistein could exert beneficial effects on endothelial function in healthy postmenopausal women.
Aim and Methods. We aimed to evaluate the effects of genistein on endothelial function in postmenopausal women with metabolic syndrome (MS). Twenty postmenopausal women with MS, according to modified NCEP-ATP III criteria were randomly assigned to receive placebo or genistein (54 mg/day) for 6 months, along with a Mediterranean-style diet. Postmenopausal women without MS (n=15), served as controls. The primary outcome was the assessment of endothelial function by flow-mediated vasodilation (FMD) of brachial artery; moreover, time to peak dilation in the FMD response has been evaluated. Secondary outcomes were fasting glucose and insulin, total cholesterol, LDL and HDL cholesterol, triglycerides, visfatin, adiponectin and homocysteine blood levels. Data on adverse events were also recorded.
Results. After six months of treatment FMD at 50s and peak FMD significantly increased in genistein recipients compared to placebo. Moreover, genistein significantly decreased the blood levels of total cholesterol, triglycerides, homocysteine and visfatin compared to placebo; while blood adiponectin levels were increased. No placebo or genistein patients discontinued treatment because of adverse events.
Conclusions. Six months of treatment with genistein effectively improves endothelial function in postmenopausal women with MS.

P2.9

Physical, chemical and morphological changes of bread polyphenol extract on cellular cultures monolayer CaCo-2

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The effect on potential transepithelial electrics resistance (TEER) and the electronic scansion microscopy analysis (SEM) of CaCo-2 cells line monolayer could be one useful method to study the different antioxidant or pro-oxidant capacity values of different concentrations of the hydrolizated polyphenol extracts of bread made with wheat Sant'Agata cultivar (HBPE).The various concentrations of HBPE have been tested on culture in monolayer CaCo-2 cells seeded on polycarbonate filter, in the cell culture chamber (Ø 6.5 µm; area 0.33cm^2; pore Ø 0.4 µm), at a density of 1.5x10^5 cells per filter and placed in a multiwells Falcon; the filter divided the chamber in apical and basal compartments that represent the lumen and the basal area of the gut tract. With HBPE concentrations of 5,10,25,50,100 µg/ml it was observed that both methods (TEER and SEM analysis) showed an antioxidant activity in the monolayer cells incubated with 5 and 10 µg/ml of HBPE, instead the 25,50,100 µg/ml concentrations of HBPE showed a TEER value decrease. This means an evident change of the permeability owed at cells damage. This is confirmed by SEM analysis, which reported a remarkable cell damages due to pro-oxidant activity of HBPE.
Conclusion: from our results it is possible to observe the influence of bread polyphenol extract concentrations. In fact, the low concentrations 5 and 10 µg/ml showed an antioxidant behaviour. Increasing the concentrations 25,50,100 µg/ml the HBPE showed a pro-oxidant behaviour.

P2.10

Metabolic responses to isoenergetic intake of cow, donkey or human milk in rats

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Aims The objectives of this study were to evaluate the effects produced by cow (CM), donkey (DM) or human milk (HM) dietary supplementation on lipid metabolism, inflammatory status and antioxidant/detoxifying defenses in rats.
Methods Three groups of rats were supplemented with isoenergetic amount of raw CM, DM or HM for 4 weeks. The fourth group (control),received only standard diet. Energy balance, HOMA index, serum levels of pro-inflammatory cytokines
and hepatic antioxidant/detoxifying enzymes, mitochondrial energy efficiency and oxidative stress were evaluated.
Results Metabolisable energy intake was increased by milk treatment, but HM and DM intake improved animal energy expenditure without any effect on body weight gain, as compared to CM-treated or to control rats. Progressive reduction of HOMA index was evidenced in differently treated animals (DM>HM>Control>CM). Significantly lower levels of pro-inflammatory cytokines were found in HM and DM-treated rats, associated to decreased body weight, lipid gain and liver lipids. Moreover, the hypolipidemic effect produced by DM or HM intake paralleled with enhanced mitochondrial proton leakage and detoxifying enzyme activities and reduced oxidative stress.
Conclusions Decreased energy efficiency with reduced pro-inflammatory signs and the improved redox status or detoxifying enzyme activities in HM or DM-treated animals, indicated that anti-inflammatory effects were attributable, at least in part, to improved cyto-protection.

P2.11

Role of ERbeta in human melanoma cells and its involvement in tocotrienols activity

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The incidence of melanoma is increasing worldwide and its prognosis is still poor. Clinical observations demonstrate that estrogen receptor-beta (ERb) is expressed in human melanoma tissues and its level of expression decreases with tumor progression, suggesting its cancer suppressive activity. Recently, several papers have shown that tocotrienols (TTs), unsaturated vitamin E compound, inhibit proliferation of different cancer cells. Some authors demonstrated that in human breast cancer cells the effects of TTs are mediated by binding to ERb. Based on this observation, experiments were performed to clarify the presence of ERb in melanoma cells, the effects of ERb activation by means of specific agonists and of TTs on cell growth and their interaction with ERb. Our data indicate that: 1)ERb is expressed in human melanoma cells; 2)ERb agonists significantly and specifically inhibit cell proliferation; 3)upon activation, ERb translocates from the cytoplasm into the nucleus confirming the classical activity of steroid receptors; 4)TTs inhibit melanoma cell proliferation and the use of ER specific inhibitor, ICI-182,780, abrogates their antiproliferative effects. Conclusions: in human melanoma cells ERb is associated with a significant antitumor effect. This receptor mediate the antiproliferative effects of the non-toxic dietary anti-cancer agents TTs, suggesting a role as a molecular target for novel therapies in melanoma. (Supported by Fondazione Banca del Monte di Lombardia)

P2.12

Evaluation of cut-off scores for sarcopenia in a clinical obese population

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Objective: The study was aimed to assess cut off scores of Muscle Mass Index(MMI) according to Body Mass Index (BMI) and age in Naples populations and to evaluate the prevalence of class I(CL-I) or class II (CL-II) sarcopenia in obese aging peoples. **Methods**:In 1815 recruited patients nutritional status was assessed and muscle mass was estimated by Janssen's equation;MMI was calculated as: MM/height^2. Sarcopenia was defined as Mean–1 Standard Deviation (CL-I) and Mean–2 Standard Deviation (CL-II). **Results:**BMI and gender-specific cut off scores were calculated.For Obese Adult (OBA) Men cut off scores were 9,6 Kg/m^2 for CL-I and 8,5 Kg/m^2 for CL-II,while for OBA Women cut off scores were 8,3 Kg/m^2 for CL-I and 7,3 Kg/m^2 for CL-II.For Normalweight Adult (NWA) Men the scores were 8,8 Kg/m^2 for CL-I and 7,9 Kg/m^2 for CL-II,while for NWA Women these were 7,4 Kg/m^2 for CL-I and 6,8 Kg/m^2 for CL-II.According to OBA derived cut off scores 146 (of 426 aging women) were CL-I and 44 were CL-II sarcopenic.44 (of 165 aging men) were CL-I and 7 were CL-II sarcopenic.The prevalence was reduced to 31 (of 426 aging women) as CL-I and 5 as CL-II sarcopenic, using the NWA derived cut off scores;moreover,only 5 (of 165 aging men) were class I and 2 were class II sarcopenic. **Conclusions:**The obese cut off scores, derived from OBA population, permit to define higher prevalence of sarcopenia in obese aging peoples;therefore, new approaches for sarcopenia are required.

P2.13

Selective modulation of intrinsic myogenic activity of peripheral diaphragmatic lymphatics by epinephrine

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Lymph propulsion within the most peripheral diaphragmatic lymphatics depends upon tissue displacements and by contraction of smooth muscle cells in the vessel wall. The aim of the present work was to investigate, in actively contracting sites, the contribution of single strokes to lymph propulsion and how this phenomenon is modulated by epinephrine. Anaesthetized rats received an intraperitoneal injection of a mixture of FITC-conjugated dextrans and TRITC-labeled microspheres (0.1-0.5 μm diameter). After passive lymphatic vessels loading, microspheres movement were video recorded *ex-vivo* in excised pieces of diaphragm kept superfused with oxygenated Tyrode's solution in a flow chamber on the stage of an upright microscope. Instantaneous and mean microsphere velocities and acceleration were derived from microsphere trajectories along with vessel diameter changes imposed by spontaneous single active strokes. Data obtained show that active strokes exert a distance-dependent effect on both velocity and acceleration of microspheres. In the presence of intraluminal valves, microspheres show an oscillatory trajectory on the proxymal side and monotonic outward directed flow on the distal side of the valve.. Epinephrine administration has opposite effects in linear vessels and lymphatic loops: in particular, epinephrine determines an increase in the frequency of contraction and a greater distance traveled by microspheres in loops, whereas it has no effect in linear vessels.

P2.14

Postnatal development of the 5-hydroxytryptamine (5-HT) signaling system in the mouse duodenum

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The gastrointestinal tract is not fully mature at birth with postnatal functional development adapting to longitudinal growth and dietary changes. 5-hydroxytryptamine (5-HT) is one of the key molecules that link the gut lumen environment to the enteric nervous system to generate adequate motility patterns. Thus, we aimed to study if 5-HT signaling system undergoes to postnatal maturation, using mouse duodenum as model. Contractile responses to 5-HT were analyzed, *in vitro*, in 2 day-old (P2) *vs* adult mice. In both preparations, 5-HT evoked a muscular contraction, being its efficacy greater in P2 duodenum. The effects were due to activation of muscular 5-HT receptors, antagonized by methysergide, nontarget 5-HT receptor antagonist, and of neural 5-HT_3 receptor, antagonized by ondasetron. In both preparations there was a major sensitivity of the postjunctional *vs* prejunctional receptors. In P2 duodenum, neural 5-HT response was abolished by atropine, muscarinic receptor antagonist. In adult duodenum, atropine revealed a muscular relaxation, antagonized by L-NAME, a NOS inhibitor. L-NAME *per se* potentiated the 5-HT cholinergic contractile effects only in the adult preparations. In conclusion, in mouse duodenum, 5-HT signaling undergoes to age-related changes. Contractile response, present from birth, decrease in efficacy, and concurrently a muscular NO-mediated relaxation occurs. These changes may contribute to gut motility adaptation to cope with the dietary changes at weaning.

P2.15

Atypical antipsychotic increases phospho-AMP-activated protein kinase in the hyphotalamus

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Antipsychotic drugs can cause body weight gain, suggesting an influence on the hypothalamic mechanisms for feeding and methabolism regulation. The aim of the present work was to study the effects of haloperidol, a typical antipsychotic drug, and olanzapine, an atypical antipsychotic, on the hypothalamic expression of AMPK, a serine-threonine kinase involved in food intake regulation. In particular the catalytic α subunit of AMPK (AMPKα), and the active form of the α catalytic AMPK subunit (p-AMPKα) were analyzed. Three groups of four rats each were treated as follows: 1) haloperidol i.p. (1mg/kg b.w.); 2) olanzapine i.p. (10mg/kg b.w.); 3) saline i.p. Insulin was then injected i.c.v. 30 min after the i.p. injection. The levels of AMPKα and p-AMPKα proteins were evaluated by Western blot, immunohistochemistry and immunofluorescence 2h after the i.p. injection.
The results showed a significant increase of pAMPKα in the animals treated with olanzapine compared to the control. pAMPKα was expressed in particular in the neurons of the arcuate and lateral hypothalamus. No significant difference was seen between the olanzapine- and saline-group on the AMPKα levels. Moreover no significant difference was between the haloperidol- and the saline-treated groups on the expression of AMPKα and p-AMPKα. This result suggests that an up-regulation of AMPK can be responsible of the weight gain caused by treatment with atypical antipsychotics.

P3.1

AngII-dependent modulation of eel heart morpho-functional remodelling

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The octapeptide Angiotensin II (AngII) is a multipotent hormone whose biological actions include short-term modulation and long-term adaptations. In the eel, AngII elicits a short-term cardio-modulatory effect. However, information regarding the influence of AngII on cardiac remodelling is lacking. We used freshwater eels (*Anguilla anguilla*) intraperitoneally injected for 4 weeks with saline or AngII (0.4 or 1.2 nmol g BW^{-1}) or AngII (1.2 nmol g BW^{-1}) plus the AT_2 receptor antagonist CGP_{42112}. Using an *in vitro* working heart preparation, cardiac performance was evaluated under loading (i.e. preload and afterload) challenges. Hearts of all groups showed similar Frank-Starling responses. However, in response to afterload increases, stroke volume rapidly decreased in control hearts, while it was better maintained in AngII-treated counterparts. These effects were abolished by an antagonist of the AT_2 receptor, whose cardiac expression was revealed by western blotting analysis. We also found by immunolocalization and immunoblotting that AngII influences both expression and localization of molecules involved in cell growth and apoptosis, such as c-kit, apoptosis repressor with CARD domain (ARC), heat shock protein 90 (Hsp90), and endothelial Nitric Oxide Synthase "(eNOS)-like" isoform.
These results point to a role of AngII in eel heart remodelling, providing new insights regarding the modulation of cardiac plasticity in fish.

P3.2

Effects of different environmental *Vibrio* strains on functional parameters of mussel hemocytes

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Marine bivalves can accumulate large numbers of bacteria, in particular *Vibrio* species, that can persist within bivalve tissues. *Vibrios* are gram-negative bacteria autochthonous of marine environment; many of these are known as an important cause of human food-borne illnesses. However, also in bivalves, continuous exposure to *Vibrios* represents a source of environmental stress. Mussels can cope with this environmental challenge through modulation of the physiological defence mechanisms against potential pathogens. On the other hand, few reports are available on responses of *M. galloprovincialis* hemocytes to environmental *Vibrio* isolates. In this work the effects of *in vitro* challenge of hemocytes with *V. parahaemolyticus* 80 and *V. vulnificus* 509 (isolated from environmental samples), were investigated in comparison with those of available reference strains, *V alginolyticus* 1513 and *V. parahaemolyticus* ATCC43996.
The results indicate differential responses to different *Vibrios* in terms of activation of immune parameters and lysosomal membrane destabilization. Moreover, the specific response of mussel hemocytes to environmental isolates *V. parahaemolyticus* 80 and *V. vulnificus* 509 was evaluated in terms of activation of p-38 MAPK and apoptotic cell ratio.
The results demonstrate the existence of differential functional immune responses in mussels to different *Vibrio* strains and indicate that mussel hemocytes can be utilized for elucidating the mechanisms for pathogen action.

P3.3

Adrenergic receptors and signaling in yellow and silver European eel hepatocytes

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European eels spend up to twenty years in fresh or brackish water as "yellow" eels. Then, part of the population change into migrant "silver" eels, moving towards the spawning grounds at the Sargasso sea. Migrating eels do not feed, and show higher levels of cortisol, causing lipolysis in muscle and liver and higher free fatty acids into the blood. Further hormones change their levels and modulate metabolism in migrating eels, including insulin, IGFs, leptin, and ghrelin. Our investigation compared for the first time catecholamine regulation of liver metabolism in yellow and silver eels. Expression of α1- and β2-adrenergic receptor (AR) mRNAs in eel hepatocytes was assessed by qRT-PCR. Levels of both α1- and β2-AR mRNAs were significantly higher in hepatocytes from silver than from yellow eels, with a 5-fold and a 2-fold increase, respectively. As a result, the mean ratio α1-AR/β2-AR transcripts was significantly higher in silver than in yellow eel. In a first approach, the β2-AR coupled signal transduction was investigated. Epinephrine dose-dependently increased cAMP levels in eel hepatocytes, an effect counteracted by propranolol. Under the same stimuli, the extent of cAMP increase was similar at the two life-stages; differently, the induced glucose release was significantly higher in silver than in yellow eels. Since α1-AR mediated pathway concurs to modulate liver glycogenolysis, further studies will evaluate the contribution of calcium signaling in silver eel response.

P3.4

Mechanims of hydrogen sulphide signalling in frog and rat hearts: Akt/eNOS phosphorilation and PLN S-Sulfhydration

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H_2S is as a novel gaseous transmitter that mediates mammalian cardiovascular homeostasis. In non-mammalian vertebrates H_2S shows both vasodilatory and vasoconstrictory properties which are species- and vessel-specific. In contrast, its cardiac effects remain almost unexplored. The purpose of the present study was to analyze the effects of H_2S on the performance of the avascular heart of *Rana esculenta*, and to compare them with those obtained on the rat heart, used as mammalian prototype. Attention was focused on the intracellular signaling with particular reference to the interaction with the NO system and S-Sulfhydration of proteins. In the frog heart, NaHS (used as H_2S donor, $10^{-12}\div10^{-7}$M) dose-dependently decreased inotropism. This effect was reduced by glibenclamide (K_{ATP} channels blocker), L-NMMA (NOS inhibitor), ODQ (guanylyl cyclase inhibitor), KT_{5823} (PKG inhibitor), and it was blocked by Akt1/2 (Akt inhibitor) and by detergent Triton X-100. In the rat, in addition to the classic negative inotropic effect, NaHS ($10^{-12}\div10^{-7}$M) exhibited negative lusitropism. NaHS treatment induced Akt and eNOS phosphorylation and an increased protein S-sulfhydration that, in the rat heart, includes phospholamban (PLN). Our data suggest that H_2S represents a phylogenetically conserved cardioactive molecule. Results obtained on the rat heart extend the role of H_2S also to cardiac relaxation. H_2S effects involve K_{ATP} channels, the Akt/NOS-cGMP/PKG pathway and proteins S-Sulfhydration.

P3.5

Nitrite effects on swimming performance in the convict cichlid, Amatitlania nigrofasciata

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Environmental nitrite is particularly toxic to fresh water teleost. Its major effect is the methemoglobin (metHb) formation and induction of functional hypoxia in fish tissues. The present study reports the effect of an acute exposure (3h) to a sub-lethal concentration of environmental nitrite (2mM) on the swimming performance of convict cichlid (*Amatitlana nigrofasciata*, leucistic phenotype), as evaluated by the critical swimming speed (Ucrit), i.e. the measure of the highest swimming speed sustained by the aerobic metabolism, determined through swimming tests with stepwise incremental speed.
After nitrite treatment, we observed, in resting animals, a strong increase in nitrite levels of blood and tissues, as well as blood methemoglobinemia (45% mtHb), indicating the occurrence of a significant functional hypoxia. Despite this, Ucrit was not affected by the nitrite treatment. However, the analysis of several tissues (white muscle, red muscle, gills, liver/intestine), showed a significant reduction of lactate levels in the animals subject to exercise compared to the sedentary ones, both in absence and presence of nitrite. These results suggest that lactate, in this species, is involved in sustaining maximal performance of aerobic swimming.

P3.6

Physiological responses to Cd exposure in *Mytilus sp.*

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It is well known that bivalves accumulate metallic pollutants at concentrations several orders of magnitude above those observed in the field environment. Among molluscs, mussels are extensively used in environmental monitoring studies.
Cadmium is a non-essential heavy metal and exhibits a nutrient-type behavior. It has a high affinity for the sulfhydryl groups of cysteine, and competes against zinc and copper for the sites of various enzymes, thus impairing their catalytic activities and enhancing lipid peroxidation. Cd may also have a direct effect on peroxidation reactions.
Metallothioneins (MTs) play a key role in detoxifying non-essential metals such as Cd, are involved in cellular antioxidant functions and also in interaction mechanisms with glutathione (GSH), that is considered the first defensive line against Cd toxicity. The enzyme glutathione reductase (GR), which is responsible for the reduction of the disulphide GSSG, is described as being inhibited by several metal ions.
In this study, effects of Cd on cellular MT, GSH content and GR activity of *Mytilus* sp. have been investigated. Mussels were stabulated in tanks containing sea water. For each time (12, 24, 48, 72, 96 hours) ten mussels exposed to 0.28 ppm cadmium and five control mussels were examined. Since the digestive gland and gills are important target organs for metal accumulation, the two tissues were chosen to investigate the biochemical responses.
The first results show that: 1) GSH and MT concentration are higher in digestive gland than in gills; 2) Cd increases defense responses in relation to exposure time.

P3.7

Effects of Hyperbaric Oxygen exposure (HBO) in patients before surgical pancreatoduodenectomy

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HBO might attenuate the production of pro-inflammatory cytochines in response to the inflammatory stimulus such surgery. Pancreaticoduodenectomy (PD) represents one of the most important surgical procedures burdened by a considerable number of local and systemic complication.The main objective of this study was to identify the possible presence of major differences between the concentration of inflammatory citokines in two study groups depending on the receiving or not HBO before PD procedure. Secondary objective was the comparison of the complication rate and hospital stay between the two study groups.The study was a prospective, randomized double-blind study lasting 6 months. Twenty-four hours before PD, Patients of group "A" were submitted to HBO session while Patients of group "B" breathed air in an hyperbaric chamber pressurized to 1.15 ATA (placebo procedure). Patients of group “A” were exposed to a single hyperbaric exposure at 2.5 ATA for a duration of 110 min. In all patients, blood was taken before (T0) and at the end (T1) HBO session or placebo procedure, in the first post-operative day (pod) (T2) and in the seventh pod (T3). Human Inflammatory Cytokines kit was used. The maximum concentration of cytokines was in T2 and HBO exposure can modulated IL-6 and IL-10 (p: 0.009 and 0.03 HBO vs placebo). Pulmonary infection is correlated only with HBO exposition (p=0.023). This paper may suggest a potential role of HBO in decreasing the incidence of pneumonie infections. HBO also can attenuate the production of pro-inflammatory cytochines in response to the inflammatory stimulus.

P3.8

Peroxiredoxin 6 and antioxidant defences in Antarctic fish

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Peroxiredoxins (Prxs) are a family of small (22 – 27 kDa) non-selenium peroxidases that are able to reduce hydrogen peroxide, organic hydroperoxides and peroxynitrite, thus representing a class of important antioxidant enzymes, that protect cells against oxidative stress. In this work we have for the first time characterized Prx6 in four Antarctic teleost species (*Trematomus bernachii*, *Trematomus pennelli, Gymnodraco acuticeps* and *Chionodraco hamatus*), and studied the transcription of this gene in different tissues, also in relation with other antioxidant system genes, such as superoxide dismutase and glutathione peroxidase. Amminoacidic and nucleotide sequences of these genes show high homology with respect to orthologs from other metazoans. The residues important for the catalysis, included into a specific tetrad for phospholipase activity and for peroxidases activity, are conserved. Our phylogenetic reconstruction confirmed the monophyletic origin of Prx6 group. The distribution of Prx6 into Antarctic teleost clade reflects the evolution of species, the two sequences of *Trematomus* (Nototheniidae family) emerging together and separated from Prx6 of *C. hamatus* and *G. acuticeps*, species belonging to Channichthydae and Bathydraconiade families, respectively. To better understand the evolution of Prx in Antarctic teleosts, other Prx sequences will be characterized and used to improve our phylogenic reconstruction. The transcription of *prx6* gene was studied in three species: *G. acuticeps*, *T. bernachii* and *C. hamatus*. Data shows that the transcription are present in all tissues, especially in muscle and heart, probably in relation to their high metabolic activity. (Grant by M.I.U.R.)

P3.9

Effect of Sodium Dodecyl Sulfate on RVD of digestive cells of *Mytilus galloprovincialis*

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Aquatic pollutants produce multiple consequences at organism, population, community and ecosystem level, affecting organ function, reproductive status, population size, species survival and thus biodiversity. Among these, detergents are a big concern reaching aquatic ecosystems by different sources. Anionic detergents such as Sodium Dodecyl Sulfate (SDS), produce their toxic effects on aquatic organism primarily by their ability to absorb onto and penetrate cell membrane.

In this work the effect of SDS at cellular level was tested by studying the effect of the detergent on RVD (regulatory volume decrease) on digestive cells of digestive gland of *Mytilus galloprovincialis*. For this purpose the mussels were exposed to two different concentration of SDS (0.1 mg/l and 1mg/l) for 10 and 18 days. The ability to perform RVD was evaluated by videometric technique in isolated cells from control and detergent exposed animal, exposed to hypotonic shock. The viability of cells was tested by Trypan blue test at the end of each experiments.

We found that the cells of the exposed animals, unlike those of the control ones, were not able to perform RVD. The impairment of the homeostatic response was observed in all the experimental conditions above mentioned. However on the application of the hypotonic stress the initial swelling exhibited a dependence on the detergent exposition time at both the concentrations tested. Moreover, the lysosomal stability (by neutral red retention method) was altered.

Our results suggest that the RVD could be useful to assess the adverse effect of sublethal concentrations of detergents at cellular level.

P3.10

Acetylcholinesterase activity and expression in PC12 cells exposed to high-frequency electromagnetic fields (GSM 1.8 GHz)

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The potential health risks of exposure to high frequency electromagnetic fields (HF-EMF) associated with mobile telephony have become a great public concern. A relevant number of investigations were performed to ascertain whether HF-EMF interact with cells to induce adverse biological effects. Acetylcholinesterase (AChE) activity was assessed as possible target of EMF in some studies, obtaining controversial results. In the present study AChE activity was assessed in PC12 cells exposed for 24 h to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR = 2 W/kg), largely used in mobile telephony. PC12 cells are a good model to study AChE because they synthesize and secrete acethylcholine and the degradative enzyme AChE. Moreover PC12 cells are widely used as a neuronal model to study molecular mechanisms mediating HF-EMF effects. A significant stimulation of enzyme activity was observed, and in particular Vmax values increased, while Km values remained unchanged, thus suggesting a possible enhancement in AChE expression. Differently from what expected, the levels of AChE transcripts evaluated by Real time-PCR were not affected by HF-EMF exposure. These results suggest an interaction between HF-EMF and the cells, since AChE Vmax is significantly increased. However, AChE gene expression remained unchanged; therefore other targets of HF-EMF, including protein expression/degradation or membrane microenvironment, need to be considered.

Abstracts

Poster Session 2

Topic 4: Cell Physiology
Topic 5: Physiology of Motor Systems and Exercise

P4.1

The physiological role of the estrogen receptor alpha ubiquitin binding surface in 17beta-estradiol-dependent cell proliferation and cholesterol homeostasis

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17beta-estradiol (E2) exerts its pleiotropic effects through the estrogen receptor alpha (ERa). The E2:ERa complex regulates several physiological processes including cell proliferation and metabolism through transcriptional and non-transcriptional membrane-initiated effects. Many post-translational modifications occur on ERa and are regulated by E2. Among others, ERa monoubiquitination (monoUb) contributes to the E2:ERa-mediated activation of the signalling pathways required for cell proliferation (*e.g.*, PI3K/AKT pathway). Monoubiquitinated proteins often possess an ubiquitin binding domain, which allows the non-covalent association to ubiquitinated binding partners. However, whether non-covalent Ub:ERa binding could occur and play a role in the E2:ERa signalling is unknown. Here, we report the *in vitro* structural identification of an Ub-binding surface (UBS) within ERa. Remarkably, the mutation of the residues, which hamper ERa:Ub-binding prevents both ERa association to ubiquitinated species in cells and ERa monoUb. Moreover, lack of ERa:Ub-binding strongly affects the E2-evoked activation of the extra-nuclear PI3K/AKT pathway, the E2:ERa dependent gene expression and, in turn, E2-induced cell proliferation and cholesterol homeostasis.
Altogether, these data indicate that the ERa UBS is important for the regulation of the E2-induced cell proliferation and lipid homeostasis and suggest that ubiquitinated proteins can regulate E2:ERa signalling to physiological functions.

P4.2

Serpinin as a novel CgA-derived cardioprotective peptide

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Serpinin (Serp) peptides derive from proteolytic cleavage of Chromogranin-A C-terminus. Three Serp forms were detected in rat heart: Serp, pyrogutaminated Serp(pGLU-Serp) and an extended form(Serp-RRG). Serp and pGLU-Serp are positive cardiac modulators, through a NO-independent β1-AR/AC/cAMP/PKA pathway. pGLU-Serp enhanced contractility more than Serp. Here we explored whether pGLU-Serp cardioprotects against ischemia/reperfusion injury. Ischemic pre- and post-conditioning(PostC) are interventions that limit reperfusion injury, reducing infarct size(IS) *via* the Reperfusion-Injury-Salvage-Kinases(RISK) pathway. In the presence of hypertension, PostC is blunted. Effects of pGLU-Serp, in pre- and PostC, on IS and cardiac function (left ventricular pressure:LVP) were studied in isolated hearts of normotensive(WKY) and hypertensive(SHR) rats. In both, pGLU-Serp induced a mild PreC cardioprotection, in contrast to a streaking PostC cardioprotection. pGLU-Serp-PostC reduced significantly IS, being more protective in SHR than in WKY. Conversely, LVP post-ischemic recovery was greater in WKY than in SHR. pGLU-Serp-PostC reduced contracture in both strains. Co-infusion with RISK inhibitors (AkT, MitoKATP, PKC) blocked pGLU-Serp-PostC protective effects. pGLU-Serp also protects H9c2 cardiomyocytes against hypoxia. Our data provide the first evidence of a Serp-PostC-induced cardioprotection, also suggesting a role for the peptide to overcome the hypertension-induced failure of PostC.

P4.3

Dynamic interplay between genes involved in carnosine and copper metabolism under carnosine exposure in neuronal and astrocytic mammalian cells

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Carnosine is a bioactive dipeptide that physiologically occurs in the CNS, and as such it is currently investigated in our laboratory for its action(s) as a homeostatic protective molecule in neurodegenerative processes. In nervous cells, carnosine levels largely depend on a complex regulation that involves biosynthesis, degradation and transmembrane transport processes. Furthermore, due to its intracellular pH buffering, antioxidant, anti-protein crosslinking, and metal chelation properties, carnosine homeostasis may be intertwined with heavy metal(s) homeostasis; in particular, with copper (Cu), which excess/deficiency leads to oxidative stress and impairment of protein function, and which levels are modulated by means of specific chelating/carrier molecules. Here, we show the effects of carnosine on the expression of genes directly involved in its own biosynthetic, degradative and transport pathways, such as *CARNS1* (carnosine synthase 1), *CNDP1* and *CNDP2* (serum and cytosolic carnosinase), *SLC15A1-4* (dipeptide transmembrane carriers), in both neuronal and astrocytic mammalian cells. Furthermore, we show that this naturally occurring dipeptide modulates expression of genes directly involved in Cu homeostasis, such as *SLC31A1*, coding for the high affinity metal importer CTR1, and its upstream transcription regulator *Sp1*. Our findings may contribute to define the metabolic network in which this natural dipeptide operates in the critical context of neurodegeneration.

P4.4

Transient kinetics measured with force steps discriminate between double stranded DNA elongation and melting and define the reaction energetics

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Double stranded DNA pulled to ~65 pN undergoes an overstretching transition from the basic conformation (B-form) to a 1.7 times longer conformation that represents a fundamental stage in the structural transitions involved in DNA recombination, replication and repair; thus understanding the mechanisms that control the relative stability of these different conformations is of basic importance. By using a dual laser optical tweezers with a fast force feedback (2 ms rise time), we recorded the length transient following 2-30 pN force steps imposed on the λ-phage DNA with different degrees of melting and at different temperatures (10-25°C). The exponential elongation following 2-30 pN force pull to the overstretching force shows that the whole 70% extension is a two state reaction with a cooperativity of 22 bp. The length transient following a 20-30 pN force drop from the overstretching force is made by stepwise shortenings due to backward two state reactions and pauses due to re-annealing of the melted fraction. The temperature dependence of the lengthening transient shows that there is an entropic contribution to the free energy change of the reaction that at room temperature is only 1/3 of the entropy change expected from thermal melting and that the cooperativity of the elementary elongation is independent of temperature, suggesting that it arises from structural factors, such as the nucleic acid sequence.
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P4.5

Cardiac effects of acute exposure to titanium dioxide nanoparticles: electrophysiological characterization

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The potential adverse effects of titanium dioxide nanoparticles (TiO_2-NPs) on cardiovascular system are still largely unknown. The aim of this study is to investigate the direct effects of acute exposure to TiO_2-NPs on cardiac electrical function, at cellular and organ level. Single ventricular cardiomyocytes were enzymatically isolated from rat hearts, brought in patch clamp whole-cell configuration in order to measure in controls and TiO_2-NPs treated cells 1) membrane capacitance (C_m), 2) membrane resting potential (V_r) and action potential duration (APD). Multiple epicardial electrograms were also recorded *in vivo* on the ventricular surface or rat hearts after 4h tracheal instillation of vehicle (control) or TiO_2-NPs solution (2mg/Kg), during normal sinus rhythm and ventricular pacing. TiO_2-NPs did not modify C_m and V_r values; however, in cells incubated with TiO_2-NPs, 30 sec recordings of V_r showed a significant increase of V_r variability. The treatment with TiO_2-NPs induced also a significant shortening in plateau and late phase of repolarization of APs. Electrophysiological parameters from epicardial mapping revealed that PQ, QRS and QT durations were significantly reduced, while excitability and anisotropy ratio were increased. In conclusion, the TiO_2-NPs induced decrease in cellular APD and in electrographic QT intervals associated with an increase of cardiac excitability, could be responsible for a cardiac substrate more prone to arrhythmia development.

P4.6

Effect of hyperbaric oxygen treatment (HBO) and gemcitabine on apoptosis in pancreatic ductal adenocarcinoma cells

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Gemcitabine is first-line therapy for advanced pancreatic ductal adenocarcinoma (PDAC) with poor survival and response rate. HBO enhances delivery of oxygen to hypoxic tumor cells and increase their susceptibility to cytotoxic effects of chemotherapy. We hypothesized that anticancer activity of gemcitabine may be enhanced if tumor cells were placed in oxygen rich environment. This study evaluated the effects of gemcitabine, HBO and their combination on apoptosis of PDAC tumor cells. PANC-1 and AsPc-1 PDAC tumor cell lines were used. Cultured tumor cells were treated with gemcitabine at its growth-inhibitory concentration (IC_{50}) value and HBO at 2.5 ATA for 90 minutes or combination of both. Twenty-four hours later, apoptotic cells in each group were analyzed and apoptotic index (AI) calculated. PANC-1 cell line: HBO alone had no effect on AI: 6.5±0.03 vs. 5.9±0.01. HBO before and after gemcitabine did not further increase AI: AI: 8.2±0.02, 8.5±0.02 vs. 8.1±0.02. Combination of HBO and gemcitabine significantly increased AI: 10.7±0.02 ($p<0.001$ vs. all groups). AsPc-1 cell line: HBO alone had no effect on AI: 5.9±0.03 vs. 5.9±0.01. HBO before and after gemcitabine did not further increase AI: 8.2±0.02, 8.4±0.02 vs. 8.0±0.01.Combination of HBO and gemcitabine significantly increased AI: 9.7±0.02 ($p<0.001$ vs. all groups). Our data show that HBO alone, or administered before and after gemcitabine has no effect on apoptosis in PDAC cells in vitro. HBO significantly enhanced gemcitabine-induced apoptosis when administered with gemcitabine therapy.

P4.7

N-ethylmaleimide sensitive fusion protein (NSF) complex formation and its biological regulation: implication for synaptic vesicle exocytosis

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ATPases Associated with various cellular Activities (AAA) constitute a large family of proteins involved in macromolecules remodeling through ATP hydrolysis. N-ethylmaleimide-Sensitive Factor (NSF) is a AAA-ATPase responsible of SNARE complex disassembling during synaptic vesicle exocytosis. NSF is a homohexamer with each monomer composed of three domains. Structural details from isolated domains and electron microscopy data on the full-length protein are limited to proteins from non-human sources. In particular, the detailed mechanism of NSF oligomerization, such as the nature of the intermediate species and the role played by ATP on the oligomerization, are yet to be defined. In addition, how NSF oligomerization, and hence its activity, is post-translationally regulated is unclear. Nitric oxide (NO) has been shown to regulate NSF activity via S-nitrosylation of key cysteine residues. However, the impact of these modifications on NSF structure remains unknown. The modulation of neurotransmitter release is also influenced by the activity of presynaptic protein kinases and phosphatases. On these premises, understanding the impact of phosphorylation on NSF activity is important to both unravel the functional basis of NSF activity and to uncover potential new pathways deregulated in neurodegenerative processes. The data presented in this study will describe the dynamic structural properties of the ATP dependent regulation of human NSF as well as the structuring effects of phosphorylation and NO-mediated S-nitrosylation on NSF.

P4.8

Spontaneous beating of guinea pig sinoatrial cells under pharmacolgical modulation of two different pacemaker mechanisms

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Two main mechanisms, one related to I_f current and the other to the intracellular calcium cycling, are proposed to underlie the diastolic depolarization (DD) phase of the action potential of sinoatrial cells (SANCs). The relative contribution of the two mechanisms is still unclear, and their regulation is of great clinical and pharmacological interest. The aim of our study was to compare the effect of 3μM Ivabradine (IVA, specific If blocker) and of the same concentration of Ryanodine (RYA, blocker, at this concentration, of sarcoplamic calcium release channels) on action potential (AP) parameters of guinea-pig SANCs, via the patch-clamp whole cell technique. Analogous studies have been previously performed on other mammalian species.
Series of APs were recorded from spontaneously beating cells, under perfusion in physiological Normal Tyrode (NT) solution at 36°C, until a steady-state (SS) AP waveform was reached; perfusion solution was then rapidly switched to IVA, and APs recorded until a new SS (3 to 5 minutes), then again to NT. The protocol was then repeated with RYA. On 7 cells, both blockers displayed, with respect to NT, comparable effects on AP parameters. In particular, they increased beating cycle length (by 27 and 30% respectively), and decreased the DD rate (by 34 and 42%). These results suggest, among other things, that each mechanism can ensure maintenance of pacemaking, in case of pathological or pharmacological downregulation of the other one.

P4.9

Cross-talk between thyroid hormone and IGF-1 in THP-1 monocytes is mediated by integrin αvβ3

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Interaction between thyroid hormones (TH) and the immune system are reported in the literature. Thyroid hormone (TH), thyroxine, acts nongenomically through mechanisms that involve a plasma membrane receptor αvβ3 integrin, a co-receptor for Insulin-like growth factor-1 (IGF-1). Previous data from our laboratory show that there is a cross-talk between TH and IGF-1 because TH inihibits the IGF-1 stimulated glucose uptake and cell proliferation in L-6 myoblasts and the effects are mediated by integrin αvβ3. Recently, IGF-1 has been shown to behave also as a chemokine, being an important factor for tissue regeneration after damage. In the present study, using THP-1 leukemic monocytes, expressing αvβ3 integrin in their cell membrane, we focused on the cross-talk between TH and IGF-1 studying cell migration and proliferation stimulated by IGF-1, and the role of αvβ3 integrin by the use of specific inhibitors of αvβ3 integrin. Our results show that IGF-1 is a potent chemoattractant in THP-1 monocytes and thyroid hormone inhibits the effect through αvβ3 integrin. Thyroid hormone also inhibits IGF-1-stimulated cell proliferation: this effect is also due to αvβ3 integrin: an example of a cross-talk between genomic and nongenomic effects of thyroid hormones. Furthermore, we studied the pathways involved in the modulation of proliferation and migration, by a pharmacological approach and our findings indicate that there is a different downstream signalling: Phosphatidylinositol 3-kinase (PI3-K) for IGF-1 and Mitogen-activated protein kinase (MAPK) for TH.

P4.10

Threonine 67 as a molecular hinge for the coupling mechanism in the NSS amino acid transporter KAAT1

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The crystallization of the bacterial homologue LeuT represents an outstanding step forward in the comprehension of the molecular physiology of Neurotransmitter:Sodium Symporters, but the molecular determinants of coupling mechanism and ion selectivity remain to be fully elucidated yet. We have here exploited the almost unique physiological features of KAAT1, namely the possibility to exploit K^+ as driver ion, the weakly chloride dependence and the ability of the driver ion to influence the substrate selectivity, to investigate such determinants. Comparison of amino acid sequences and structural modelling highlights in KAAT1 the presence of Thr 67, since the residue is not conserved among NSS members but present as threonine only in KAAT1 and in the homologous CAATCH1 and resides in a keystone position, bridging the two sodium binding sites of the protein. Mutants of Thr 67 were expressed in *Xenopus* oocytes and functionally characterized. The mutation into tyrosine led to a protein with an uptake activity comparable to that of wt but fully chloride independent and with an enhanced stereoselectivity. Interestingly, although having unaltered affinity for sodium, the mutant showed deeply reduced transport-associated currents, revealing the uncoupling of Na^+ and amino acid fluxes. Thr 67 appears to act as a molecular hinge for the coupling mechanism, probably participating in the conformational changes that allows the cotransport of Na^+ and of the amino acid.

P4.11

Epidermal growth factor induces MUC3A and MUC5AC mucin expression via dual oxidase-2 dependent reactive oxygen species production in human enterocyte-like cells

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Epidermal growth factor (EGF) induces the expression of mucins, the main constituents of intestinal mucus layer, thus exerting a crucial role in intestinal epithelial barrier maintenance. To investigate the molecular mechanisms underlined, we evaluated the involvement of Dual Oxidase 2 (DUOX2) enzyme on EGF induction of intestinal mucin expression. DUOX2, expressed at level of the gastrointestinal tract epithelium, is a member of the ROS-generating cell membrane NADPH oxidase (NOX) family involved in mucosal innate immunity. Using the enterocyte-like Caco-2 cells, we found that EGF induced either the transmembrane MUC3A and the secreted gel-forming MUC5AC mucin mRNA levels. The MEK inhibitor PD98059, the PKC inhibitor bisindolymaleimide (BIM) and the antioxidant N-acethylcysteine (NAC) prevented the EGF induction of mucin mRNAs. In addition, hydrogen peroxide induced mucin mRNA levels. Thus we found that EGF and hydrogen peroxide induced DUOX2 protein and mRNA levels. Knocking down of DUOX2 with siRNA prevented the EGF induction of MUC3A and MUC5AC mRNA levels, demonstrating the role of ROS generated by DUOX2 in the cell signaling involved in mucin expression modulation.
These data unravel a new physiological role of DUOX2 protein in the gut that, in addition to its innate host defense function, provides the luminal protection of the gastrointestinal tract mediating the induction of mucin expression by growth factors.

P4.12

Does apoptosis affect the muscle regeneration in human aged satellite cells?

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Adult skeletal muscle retains a remarkable capacity of regeneration and repair after injury, mainly through muscle-specific stem cells, known as satellite cells (SC). However, the sarcopenic process developing with ageing has been linked to an impaired regeneration: SC derived from elderly subjects are unable to carry out an adequate program of differentiation to maintain the muscle mass in a stable and functional status. Several studies have suggested that both the factors, one intrinsic to the cells themselves, and the niche, in which SC lie, mutually influenced each other. This reduced capability seems due to an impairment of myoblasts differentiation and an alteration of gene profile. In particular, specific gene pathways related to modulation of miRNAs and involved in muscle remodeling are entailed. Moreover, we also highlighted the involvement of altered ROS homeostasis along with the antioxidant activity reduction.This evidence was confirmed by our results using microarrays platform and analysis showing an age-related deregulation of some genes involved in oxidation management and in protein balance (Polimerase K, SHC1 and FOXO1A). The last two genes could also be implicated in apoptotic process activation.Further RT-PCR analysis demonstrated an alteration of differentiation and apoptotic processes and miRNAs expression. Our data indicate apoptosis as one of the possible mechanism responsible for the impaired SC differentiation and muscle regeneration in the elderly.

P4.13

Role of connexin 43 in the apoptosis induced by psychosine in mouse oligodendrocyte precursors

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Connexins (Cx) are a multigene family of structurally related transmembrane proteins with a common membrane topology able to modulate essential cellular processes such as proliferation, differentiation and migration. Among them, Cx43 is the most predominant isoform in many cell types, including neural progenitor cells; it plays a key role in programmed cell death. Up today, no study was performed for evaluating Cx43 expression in myelin-forming cells in Krabbe disease, a sphingolipidosis caused by the genetic deficiency of the enzyme β-galactosylceramidase (GALC), that hydrolyzes specific galactolipids, including galactosylceramide and psychosine. The loss of GALC results in the progressive accumulation of psychosine, demyelination, and early death. The aims of this study were to investigate Cx43 basal expression in control mouse- and twitcher - derived oligodendrocyte progenitors, and analyze its changes during psychosine-inducted apoptosis. Our results demonstrated that Cx43 expression is involved in survival of oligodendrocyte precursors and the increase of some apoptotic markers (caspase-3, Bax/Bcl2) and a downregulation of the antiapoptotic/proinfammatory NF-kB pathway are related to Cx43 levels. Taken together, these finding suggest a new Cx43-dependent molecular mechanism for psychosine-mediated oligodendrocyte progenitors death, and could open unexplored perspective for other demyelinating disease.

P4.14

Prosurvival communication between endothelial cells and mesenchymal stem cells through exosomes containing HIF1-alpha

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The transcription factor hypoxia-inducible factor 1 alpha (HIF1-a) is a downstream regulator of hypoxia response and neovascularization in adult myocardium. As exosomes mediate cell-cell communication in many stress responses, we investigated the release of exosomes containing HIF1-a (HIF1a-exo) by arterial endothelial cells (pAOECs) and mesenchymal stem cells (pMSCs), which characterize the myocardial angiogenesis. We also evaluated the transport of HIF1a-exo between the two cell lines under normoxia and hypoxia. Up to 72h of normoxic monocolture, the release of HIF1a-exo by pAOECs did not change compared to baseline, yet HIF1-a expression increased by 80±5% ($p<0.001$); although, HIF1-a espression and HIF1a-exo secretion by pMSCs were lower than pAOECs, but higher at 72h than baseline ($p<0.05$). At 72h of normoxic co-culture, the level of HIF1α-exo was similar to single-pAOECs culture and increased by 175±11% compared to baseline ($P<0.001$), even if HIF1-α level in pAOECs was reduced by 40±7% than pMSCs ($p<0.05$). The number of both cell lines was significantly higher in co-culture compared to each monocolture. At 72h of hypoxic co-colture, the number of pAOECs and pMSCs was respectively reduced by 44±5% ($p<0.001$) and 32±2% ($p<0.001$). Even if HIF1-a expression was higher in co-cultered pMSCs, the release of HIF1a-exo was lower than hypoxic monoculture and similar to normoxia. In conclusion, the cross talk between pAOECs and pMSCs through HIF1a-exo is attenuated under hypoxia.

P4.15

Influence of conditioned medium from neuroblastoma B104 or olfactory ensheathing cells in the differentiation of human adipose stem cells into neural phenotype

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The aim of this study was to verify if conditioned media (CM) obtained from olfactory ensheathing (OECs) or B104 neuroblastoma cells were capable of inducing differentiation of adipose tissue-derived mesenchymal stem cells (AT-MSCs) to a neuronal phenotype. The mammalian olfactory system is one of the few areas of the central nervous system able of continuous neurogenesis throughout lifetime supported by OECs, a source of several trophic factors. On the other hand, B104 neuroblastoma cells are recognized to induce differentiation of neural stem cells into oligodendrocyte precursor cells. Immunocytochemical procedures and flow cytometry analysis were used. Some neural markers, as nestin, protein gene product 9.5 (PGP 9.5), microtubule-associated protein 2 (MAP2), glial fibrillary acidic protein (GFAP), and neuron cell surface antigen (A2B5) were examined 24 hours and 7 days after the treatment. The results show that AT-MSCs treated with either medium express markers of progenitor and mature neurons (nestin, PGP 9.5 and MAP2) in time-dependent manner. They display morphological features resembling neuronal cells, and result negative for GFAP and A2B5, astrocyte and oligodendrocyte markers, respectively. This study demonstrates that AT-MSCs can be influenced by the environment toward a neuronal phenotype. This culture system may offer many advantages as potential material for replacement therapy in central nervous system degenerative diseases.

P4.16

Role of GAP-43 in myogenesis: the model of myogenic satellite cells from GAP-43 knockout mice

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The Growth Associated Protein 43 (GAP-43) is involved in neuronal plasticity during development and regeneration. Our recent data revealed that this protein is expressed in both myoblasts and myotubes, and its cellular localization changes dramatically during differentiation. In adult fibers, GAP-43 localization is found nearby the calcium release units suggesting a functional role for this protein.
The aim of this study is to define the role of GAP-43 during myogenesis and in myotubes. This was obtained analyzing the main cellular properties and intracellular calcium signaling of myogenic satellite cells isolated from wild type and GAP-43 knockout hetero- and homozygous mice.
The results showed similar proliferative and differentiative properties (assayed by morphological analyses) in all tested models. All myotube populations were responsive to KCl or caffeine with intracellular calcium increases. Interestingly, the myotubes from GAP-43 knockout hetero- and homozygous satellite cells, showed different intracellular calcium dynamics in respect to wild type ones. In particular GAP-43 knockout homozygous mytubes showed high amplitude spontaneous waves differently from the significantly lower ones expressed in wild type myotubes.
These data suggest that the absence of GAP-43 could not affect the myoblast differentiation and myotube formation, but it had a significant role in modulating intracellular calcium handling.

P4.17

Contribution of aquaporins and TRPV4 to astrocyte cell volume regulation

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Regulatory Volume Decrease (RVD) is a process by which cells restore their volume when swollen by hypo-osmotic stress. In this study, we have focused on the role played by two different Aquaporins (AQPs), AQP4 and AQP1, in mediating Ca^{2+} signaling after hypotonick shock and in triggering RVD, together with the Transient Receptor Potential Vanilloid 4 (TRPV4), a Ca^{2+}-permeable channel activated by the membrane stretching. Using biophysical techniques to measure the water plasma membrane permeability of WT and AQP4 KO astrocytes and of cells transfected with AQP4 or AQP1, we showed that both AQPs play a key role in RVD by affecting the initial kinetics of the swollen phase that is faster and higher in amplitude in the presence of AQPs. By calcium imaging we show that AQP4 and AQP1 mediated cell swelling significantly increases the amplitude of Ca^{2+} influx inhibited by the TRPV4 inhibitors, Gadolinium (Gad) and Ruthenium Red (RR). Finally, the effect of Ca^{2+} influx through TRPV4 on the cell volume regulation was analyzed by measuring RVD in the presence of Gad and RR or removing the external Ca^{2+}. Our results show that the RVD kinetic was unchanged in all these conditions, indicating that the TRPV4 mediated Ca^{2+} influx does not play a role in RVD. All together these results show that 1) AQPs play a key role in mediating Ca^{2+} signaling after hypotonic shock together with TRPV4, 2) AQPs are the main trigger for RVD, and 3) $Ca^{2+}$is not fundamental for RVD to occur.

P4.18

Estrogen receptor subtypes are differently requested for E2-neuroprotective effects

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The brain is one of the most important targets for estrogen actions. Indeed, the sex steroid hormone 17-β estradiol (E2) is responsible for neuroprotection against oxidative stress-induced apoptosis via its receptors. The mechanisms at the root of these effects and the involvement of the two estrogen receptor subtypes (i.e., ERα and ERβ) are presently unknown. Recently, we reported that E2-induced neuroglobin upregulation, a neuroprotectant protein, is at the root of E2 protective effects against neuronal apoptosis and mitochondrial damage. Huntingtin (Htt) is another neuron survival protein involved in several cellular processes including vescicle trafficking embryonic development, apoptosis inhibition, and transcriptional regulation. At the present, the possible effect of E2 in modulating Htt levels is completely unknown. The results obtained show that E2 induces a dose dependent increase of Htt levels in the neuroblastoma cell line SK-N-BE. This effect is detectable by 6 hours until 24 hours of E2 treatment. Intriguingly, E2-induced Htt upregulation requires the action mechanisms of ERα subtype; whereas, E2-induced neuroglobin upregulation is mediated by ERβ. As a whole these data highlights the widespread involvement of ER subtypes in E2-induced neuroprotection.

P4.19

Deregulation of cholesterol biosynthetic pathway in Rett syndrome

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Cholesterol (Chol), the principal sterol synthesized by animals, has many essential roles in cell physiology thus it is not surprising that perturbation in cholesterol homeostasis is linked to pathologies. Patients with Rett syndrome (RTT), a central nervous system development disorder, have altered plasma lipid profile with increased levels of both high density lipoprotein (HDL) and low density lipoprotein (LDL). Moreover, increased oxidative stress and decreased protein levels (65%) of Scavenger Receptor B1, which are involved in Chol lipoprotein uptake have been observed in RTT patients. These evidences suggest that an altered Chol homeostasis exists in RTT. The present work aimed to further investigate the protein network of Chol homeostasis maintenance in RTT using as a model freshly fibroblasts isolated from both, RTT and healthy donors.
The results showed that the protein levels of HMG-CoA reductase (the rate limiting enzyme of cholesterol synthesis), LDL receptor (LDLr, involved in mediating LDL uptake) and nuclear Sterol Regulatory Element Binding Proteins (nSREBPs- the transcription factors of genes committed to Chol metabolism) were significantly increased in RTT while plasma PCSK9 (Proprotein convertase subtilisin/kexin type 9, involved in LDLr degradation) was clearly decreased in RTT.
These data indicate that the protein network of Chol homeostasis maintenance is deeply affected in RTT suggesting that Chol could contribute to the pathogenesis of the disease.

P4.20

Response of cardiac muscle cells to stressful stimuli: BAG3 protein modulation

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BAG3 is expressed at high levels in skeletal and cardiac muscle *in vivo*. Oxidative stress leads to the catecholamines release by the nervous system; high concentrations of circulating adrenaline could be found after ischemic episodes. These findings have led us to assess whether the induction of BAG3 protein could also occur following treatment with adrenaline. Furthermore, considering its effect on cAMP levels raise and its use as promoter of contractile activity in cardiomyocytes, we have tested caffeine too. In particular, we found that epinephrine or caffeine *in vitro* increase BAG3 expression in cardiac muscle cells.
BAG3 localization in Z-discs has driven us to investigate an alteration of actin's structure in stimulated HCM and H9c2 cells. Immunofluorescence data have shown actin's depolymerization in adrenaline treated H9c2 and caffeine treated HCM. Moreover, flow cytometry analysis revealed a greater increase in the levels of intracellular Ca^{2+} in treated and *bag3* siRNA-transfected cells.
These data indicate that, in our experimental system, treatment with caffeine or adrenaline results in actin depolymerization and alteration of calcium homeostasis. Both phenomena could be associated to a pre-apoptotic state. Increased BAG3 expression following the treatment with adrenaline or caffeine could indicate an attempt to cell survival, since the further increase in Ca^{2+} flow following the *bag3* silencing. Future studies will be marked in order to verify if longer times of exposure to these substances may result in apoptotic cell death.

P4.21

Cardioprotection by ischemic (I-PostC) and pharmacological postconditioning (P-PostC) involve S-nitrosylation (SNO) of mitochondrial proteins: role of RISK and SAFE pathways

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I-PostC and P-PostC may trigger RISK- and SAFE-pathways, which converge on mitochondria influencing their function. Since NO is an important signaling messenger in I-PostC, we investigated whether protein SNO occurs in I-PostC hearts and whether P-PostC with Diazoxide (DZO) elicits similar effects on SNO and cardioprotection.
In isolated rat hearts, we studied the effects of I-PostC and P-PostC by DZO on *a)* kinases of RISK- and SAFE-pathway, *b)* SNO of mitochondrial proteins and *c)* reduction of death signals (PKCδ, cleaved-caspase3 and Beclin1) in cytosolic and mitochondrial fractions. Hearts underwent *1)* perfusion without ischemia (Sham), *2)* ischemia/reperfusion (I/R, 30-min/2-hours), *3)* I-PostC (5 cycles of 10-s R and 10-s I after the 30-min I), *4)* P-PostC (DZO 30 μM) or *5)* I-PostC+MPG or P-PostC+MPG. We found that I-PostC and P-PostC significantly reduced infarct size and are able to increase cellular SNO levels (WB/biotin switch assay). Importantly, I-PostC and P-PostC significantly increased SNO levels in mitochondrial fractions, including VDAC that has a cysteine residue that can be nitrosylated and may play a critical role in the mPTP formation. Moreover direct targeting of mitochondria with DZO *a)* activates the RISK-pathway *via* a redox signaling (anti-oxidant MPG avoids RISK activation) and *b)* decreases signals of death. Finally, phospho-STAT3 translocation is induced by I-PostC, but not by P-PostC, thus suggesting a redox-independent mechanism in the SAFE-pathway.

P4.22

The glutamate signalling in islet of Langerhans: molecular mechanisms of modulation

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Increasing evidence suggests that the excitatory neurotransmitter L-glutamate functions as a modulator in the islet of Langerhans, an endocrine organ involved in blood glucose homeostasis. It is release by α-cells and, by acting on specific receptors, it modulates hormone secretion and β-cell mass. Its extracellular concentration is mainly controlled by the glutamate transporter GLT1 which is expressed on the plasma membrane of β-cells (Di Cairano et al, JBC 2011; 286: 14007). Aim of the proposed research was to verify the impact of acute and chronic changes in glucose concentrations on GLT1 localization/function and glutamate signalling in the islet.
We found that acute exposure of human and clonal β-cells to high glucose concentrations (15 mM glucose) inhibited the GLT1 transport activity measured by [^{3}H]D-glutamate uptake, in a dose-dependent manner. Furthermore, total internal reflection microscopy experiments performed on β-cells transfected with a GFP-GLT1 tagged transporter demonstrated that glucose stimulation decreased the surface stability of GLT1 and increased its endocytosis. Chronic exposure of human and clonal β-cells to high glucose concentrations caused the GLT1 relocalization in degradative compartments and significantly reduced its total expression. Interestingly, a similar intracellular GLT1 staining was detected in human islets from type 2 diabetic donors (n=8) but not from healthy controls (n=5).
Understanding the molecular mechanisms that control glutamate release and signalling in islet of Langerhans may be important to control glucose homeostasis in health and disease.

P4.23

MASER-12 suborbital space flight mission: effects of altered gravity on signal transduction in primary human T lymphocytes

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In MASER-12 sounding rocket mission we investigated the influence of altered gravity on key proteins of ConA/CD28-activated human T lymphocytes. The hypergravity phase during the launch resulted in a down regulation of the IL-2 and CD3 receptor and reduction of tyrosine phosphorylation, p44/42-MAPK phosphorylation and histone H3 acetylation, whereas LAT phosphorylation was increased. Compared to the baseline situation at the point of entry into the microgravity phase, CD3 and IL-2 receptor expression at the surface of non-activated T cells were reduced after 6 min of microgravity (mg). Importantly, p44/42-MAPK-phosphorylation was also reduced in mg. In activated T cells, the reduced CD3 and IL-2 receptor expression recovered significantly during in-flight 1g conditions, but not during mg. b-tubulin increased significantly after onset of mg until the end of mg, but not in the in 1g. This study suggests that key proteins of T cell signal modules are not severely disturbed in mg. Instead, it can be supposed that the strong T cell inhibiting signal occurs downstream from membrane proximal signaling, such as at the transcriptional level as described recently. However, the MASER-12 experiment could identify signal molecules, which are sensitive to altered gravity, and indicates that gravity is obviously not only a requirement for transcriptional processes as described before, but also for specific phosphorylation/dephosphorylation of signal molecules and surface receptor dynamics.

P4.24

Adenosine receptors modulate the autocrine nAChR-driven $[Ca^{2+}]_i$ spiking activity of *in vitro* contracting myotubes

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During spontaneous contraction, developing myotubes release an acetylcholine (ACh)-like compound and adenosine. The autocrine activation of nicotinic ACh receptors (nAChRs) drives $[Ca^{2+}]_i$ spikes that characterize the *in vitro* myogenesis. Adenosine receptors (ARs) are expressed in many cells, but their role remains unclear in immature skeletal muscle. We investigated a possible interplay between AR-mediated activity, nAChRs and $[Ca^{2+}]_i$ spikes of contracting myotubes. Single channel recordings of autocrine-activated nAChR-channels showed a significant increase in mean open time and open probability when cells were treated with non-specific AR agonist (NECA). Non-specific AR antagonist CGS15943 and adenosine deaminase (ADA) produced the opposite effect. Ca^{2+}-imaging experiments revealed a 65% increase of $[Ca^{2+}]_i$ spiking activity in NECA, a 98% block in CGS, and a 88% block or decrease in ADA. AR density was measured by means of radioligand binding and preliminary results revealed the presence of A_1, A_{2A} and A_3-type ARs. When cells were treated with specific AR-subtype antagonists, it was found that the A_{2B} subtype was the main one responsible for the modulatory effect of adenosine on nAChR-channel activity. Our results indicate that AR activation promotes the spontaneous $[Ca^{2+}]_i$ spiking activity by modulating the activity of nAChR channels. We suggest that during differentiation, ARs could play a crucial role in maintaining the myotube trophism before the arrival of the nerve.

P4.25

Seeing is believing: evidence of a novel cytoplasmic structure containing functional proteasome and inducible by cytokines/trophic factors

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Background: A variety of ubiquitinated protein-containing cytoplasmic structures has been reported, from aggresomes to aggresome-like induced structures/sequestosomes or particle-rich cytoplasmic structures (PaCSs) that we recently described in some human infectious, neoplastic and genetic diseases. The different structures share some cytochemical characteristics, however their respective morphological patterns and functional roles remain largely unknown thus jeopardizing their univocal identification.
Results: We found that PaCSs resulted from proteasome and polyubiquitinated protein accumulation into well-demarcated, membrane-free, cytoskeleton-poor areas enriched in highly soluble polysaccharides. By analyzing living cells under confocal microscopy and correlative confocal/electron microscopy, we found that proteasome chymotrypsin-like activity was concentrated in cytoplasmic structures identified as PaCSs by ultrastructural morphology and immunocytochemistry of the same cells. PaCSs differed ultrastructurally and cytochemically from sequestosomes. In human dendritic or natural killer cells, PaCSs were induced by cytokines/trophic factors during differentiation/activation from blood progenitors.
Conclusions: PaCS is a novel distinctive cytoplasmic structure where polyubiquitinated proteins and functional proteasome accumulate. PaCSs seem to play a critical role in the ubiquitin–proteasome system response to immune, infectious and proneoplastic stimuli.

P4.26

Involvement of Aquaporin-4 in modulation of glucose transport and glycogen content in fast-twitch muscles in a Calcium-dependent pathway

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The absence of Aquaporin-4 (AQP4) water channel in the skeletal muscle (SM) alters the expression of proteins involved in energy metabolism and calcium handling. In this study we investigated whether alterations in Ca^{2+}homeostasis and glycemic balance actually occur in AQP4-null mice (KO) SM. Spectrofluorimetric analysis on isolated EDL fibers revealed an increase of resting cytoplasmatic Ca^{2+}, sarcoplasmic reticulum Ca^{2+} content and Ca^{2+}influx through sarcolemma in KO mice. Large glycogen storage found in KO quadriceps by enzymatic assay suggested an increased glucose transport likely due to glucose transporter GLUT4. Western blot analysis on fed mice proved increased expression of GLUT4 in KO mice. However, qPCR did not display any difference in GLUT4 mRNA copy number between wild type (WT) and KO mice. Immunofluorescence analysis carried out on isolated EDL fibers revealed an exclusive GLUT4 localization along sarcolemma in KO mice. These results provide evidence that in KO mice, GLUT4 increase is due to an up-regulation of transporter translocation to the sarcolemma. Finally, Western blot analysis on fast SM of fasting and trained WT e KO mice allowed to exclude the involvement of insulin and exercise-dependent pathways regulating this phenomenon. On the basis of our experimental data we postulate that AQP4 may modulate glucose transport and glycogen content in fast-twitch muscles through changes in Ca^{2+} concentration at the subsarcolemmal region.

P4.27

Solid Lipid Nanoparticles: a strategy to overcome the blood-brain barrier

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Diagnosis and therapy of brain diseases are often compromised by the difficulty to cross the blood brain barrier (BBB). Recently, the emerging field of nanotechnology has generated new promises to solve this problem. Nanoparticles (NPs) have several advantages in terms of biocompatibility, non-immunogenicity, non-toxicity and they can be functionalized to carry imaging agents and/or drugs, and to enhance the blood circulation residence time. Finally, the NPs surface can be modified with specific ligands in order to achieve site-specific delivery and successful penetration of the BBB. The objective of present investigation was to study the effect of surface characteristics of solid lipid nanoparticles (SLN) covalently coupled with the monomer of ApoE-residues (141-150) on cellular uptake in brain capillary endothelial cells. Radiolabelled and fluorescent (fluoroprobe strictly associated to SLN) have been used to evaluate the transcellular transport in in vitro BBB model based on human cerebral microvascular endothelial cells (hCMEC/D3). SLN loaded with different fluorescent dyes and functionalized with phosphatidic acid (Aβ ligands) and DSPE-PEG(2000)-Maleimide have been investigated. SLN uptake was monitored by confocal-laser-scanning microscopy and quantified by radiochemical techniques. The peptide mediated an efficient cellular uptake of SLN. SLN without surface-located peptide displayed less membrane accumulation and cellular uptake. These results indicate that the formulations herein analyzed are suitable tools for brain targeted drug and contrast agent delivery.
Keywords: solid lipid nanoparticles, ApoE peptide, microvascular brain capillary endothelial cells.

P4.28

Effects of acute exposure to titanium dioxide (TiO_2) nanoparticles on ventricular cardiomyocytes: mechanical and cytotoxic characterization

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There is clinical and experimental evidence that exposure to titanium dioxide (TiO_2)-nanoparticles (NPs) increases the occurrence of cardiovascular diseases, although the underlying mechanisms remain incompletely defined.
To address this issue, we investigated the effects of TiO_2-NPs on cell mechanics, oxidative stress and genotoxicity, in isolated rat ventricular cardiomyocytes.
TiO_2-NPs were suspended in sterilized water, sonicated to limit particle aggregation and then added, at a concentration of 50mg/mL, to the myocyte suspension (low-calcium solution). Atomic force microscopy showed a poor TiO_2-NP aggregation in solution with a 72% of single NPs, which measured less than 100 nm in diameter.
Cell mechanics were assessed by measuring: average diastolic sarcomere length, fraction of shortening (FS), and maximal rates of shortening and re-lengthening (±dL/dt). The generation of reactive oxygen species (ROS; DCFDH (2′,7′-dichlorodihydrofluorescein)-fluorescence assay) and DNA damage (comet assay) were also determined in control (C) and TiO_2 (T) treated cells.
In comparison with C, T myocytes exhibited a reduced FS, a significant decrease in ±dL/dt, and a higher incidence of spontaneous contractions. ROS production and genotoxicity were also increased, supporting the hypothesis of an oxidative DNA damage driven by ROS generation.
In conclusion, TiO_2-NPs worsened cell mechanics, increased ROS production and induced genotoxicity.

P4.29

Preliminary study of gene expression of TJP1, CLDN2 and MYO9B in patients with Systemic Nickel Allergy Syndrome (SNAS)

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Nickel is a metal widely distributed in nature. It is contained in many everyday objects and is the leading cause of Allergic Contact Dermatitis. In susceptible individual oral intake of nickel is able to elicit gastrointestinal disorders, atypical systemic manifestations, and chronic dermatological symptoms that are called Systemic Nickel Allergy Syndrome (SNAS). It is known that the gastrointestinal tract is the main system through which non-self antigens enter into the body. Therefore, it is possible to hypothize that any alteration of the intestinal permeability could increase the exposure to nickel in genetically susceptible individuals. According to this hypothesis, the aim of the present work was to study the relative expression of some genes involved in the functioning of tight junctions in duodenal biopsies of SNAS patients. The study focused on TJP1, CLDN2 and MYO9B, coding respectively for zonula occludens-1, claudin-2 and myosin-9b. Real time PCR was applied.
Analyzing the relative expression levels of the three selected genes, TJP1 showed comparable levels to control subjects, while MYO9B and CLDN2 appeared up-regulated in SNAS patients.
In conclusions results disclose a de-regulation of proteins involved in the functioning of tight junctions in SNAS patients, and suggest the involvement of claudin-2 and myosin-9b in the potential alteration of the permeability of the gastrointestinal barrier.

P4.30

A role for the ubiquitin-activating enzyme in 17β-estradiol-induced cell proliferation, migration and cholesterol homeostasis

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17b-estradiol (E2) binds to the estrogen receptor (ER) a and regulates diverse physiological functions including cell proliferation and migration, lipid and glucose homeostasis. We recently demonstrated that ERa is an ubiquitinated protein. ERa ubiquitination serves proteolytic and non-degradative functions but the knowledge of the interplay between E2:ERa signalling and the ubiquitin-system in the control of E2-dependent cellular functions remains primordial. The development of the 26S proteasome inhibitors revealed that many physiological functions are regulated by ubiquitination (e.g., cell proliferation and migration). Recently 4[4-(5-nitro-furan-2-ylmethylene)-3,5-dioxo-pyrazolidin-1-yl]-benzoic acid ethyl ester (Pyr-41) has been identified as the first inhibitor of the ubiquitination cascade. Thus, we determined the effect of Pyr-41 in E2:ERa signalling to cell proliferation, migration and cholesterol metabolism. By employing this novel tool, we report that the inhibition of the ubiquitination cascade fastens E2-induced ERa degradation, prevents E2-evoked PI3K/AKT and p38/MAPK activation and thus blocks E2-induced cell proliferation, migration and cholesterol metabolism. Altogether, these data point to a central role for ubiquitination in E2-regulated cellular processes.

P4.31

Insights into the mechanisms underlying copper absorption in human endothelial cells

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Angiogenic abnormalities, definitely considered a hallmark of both solid and haematological tumours, are being increasingly implicated in the pathogenesis of common neurodegenerative diseases (e.g. Alzheimer, SLA). Defective copper (Cu) metabolism is known to favour the angiogenic pathology, but the design of Cu-targeted therapeutic strategies to slow down the blood vessel deterioration is hampered by the incomplete knowledge of the vascular metal management. To contribute to profile the endothelial pathways of Cu acquisition, a functional study has been conducted in a human macrovascular cell model (HUVEC). The substrate reduction through a yet unidentified membrane cupric reductase activity has been documented as a rate-limiting step in the absorption of Cu from histidinates through the CTR1 high-affinity channel, whose activity was silver-sensitive. Outcomes from competition assays in the presence of excess free divalent cation resulted compatible with the transport activity mediated by NRAMP2 protein (order of inhibition: Zn > Mn). A “non-canonical” chloride-sensitive transport mechanism, inhibited by high extracellular bicarbonate, was also evidenced, suggesting the involvement of an anion exchanger in the absorption of chloride-complexed Cu. These results, together with available literature on micro environment-driven Cu distribution within tissues affected by vascular lesions, may help delineating some of the main “actors” of pathological blood vessel remodelling.

P4.32

Epithelial barrier impairment in HRPE cells is prevented by Goji berry extracts: cAMP as a potential trigger molecule

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Human retinal pigment epithelium (HRPE) cells are a useful model to investigate *in vitro* the high glucose-induced barrier disruption occurring in diabetic retinopathy. A potential protective effect of extracts from Goji (*Lycium barbarum*, LB) berries and their main component taurine has been previously reported, but the underlying mechanisms still await a proper elucidation. HRPE cells grown in Millicell 12-well inserts were treated for 48 h in the presence of a factorial combination of normal (5 mM) or high (25 mM) glucose, 1 mg/ml LB extract and taurine, then the Transepithelial Electrical Resistance (TEER) was measured. Cells were collected to determine intracellular cAMP levels, and the activity of adenylate cyclase (AC) was assayed on membrane and cytosolic fractions. TEER was significantly reduced by high glucose (40±8 $\Omega \cdot cm^2$), but recovered to physiological values (95±10 $\Omega \cdot cm^2$) if LB extract or taurine were also present. The direct activator of tmAC forskolin (FSK) at 1µ M reduced TEER, and the effect was partially counteracted by LB extract. Intracellular cAMP levels increased under high glucose conditions as a consequence of higher activity of the FSK-insensitive cytosolic AC. The treatment with either LB extract or taurine restored control levels. Data suggest that LB extract and taurine protect from HRPE cells barrier impairment by counteracting the glucose-induced increase of cAMP levels.

P5.1

Effects of whole body oscillations on cardiovascular variables and spontaneous variability

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Heart Rate Variability (HRV) has been well assessed as a predictor of cardiovascular morbidity and mortality, but its origin and physiological interpretation is still debated. In the present research we verified the hypothesis that HRV may be influenced by whole body forced oscillations. Experiments were performed on 7 volunteers who rested quietly on a motorized swinging table during 4 different oscillation trials. We recorded 6 min supine, 10 min oscillation (± 10° or ± 20°, 0.10 Hz or 0.05 Hz), 6 min supine. The subjects were instrumented with a cuff for beat-to-beat finger arterial pressure recording (Portapres, TNO), which also allows for calculation of several cardiovascular variables. We obtained simultaneous records of: systolic/diastolic pressure, heart rate, stroke volume, cardiac output, total peripheral resistance and hydrostatic level (measuring the vertical distance between the finger cuff and the heart and used to analyze the oscillation characteristics). Because of high variability of frequency-related data, mean changes are not meaningful, but analysis of single comparisons between pre and post oscillations allows to observe that: 1) heart rate was slightly reduced, 2) LF frequency was slowed, 3) VLF frequency was clustered around 0.05 Hz, 4) the percentage of HF was lower. These results were not different in the 4 trials. We conclude that 10 min forced oscillations is enough to influence the spontaneous frequency of HRV, but not the power of oscillations.

P5.2

Crossbridge properties during fatigue and recovery in mammalian intact skeletal muscle fibres at physiological temperature

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Fatigue occurring during exercise can be defined as the inability to maintain the initial force or power output. Fatigue during repetitive tetanic stimulation at 24°C occurred in two phases: an initial one (P1) during which individual crossbridge (CB) force decreased, followed by a later phase (P2) during which also CB number decreased (Nocella et al. *J Physiol,* 2011). The present experiments were made a) to compare fatigue at 24°C and 35°C, the *in vivo* temperature during an intense exercise and b) to investigate force recovery from fatigue. Force and stiffness were measured during a fatiguing protocol of 105 tetani at 1.5/s on fibres from FDB mouse muscle. The results showed that tension decline during P1 was smaller and slower at 35°C than at 24°C, whereas during P2 was greater at 35°C so that total force depression was the same at both temperatures. Initial force decline was accompanied by a smaller stiffness decline at both temperatures, whereas stiffness decreased in parallel with tension during P2. Similarly to fatigue, force recovery occurred in two phases: P1 was associated with the recovery of individual CB force, whereas P2 was mainly due to the recovery of the pre-fatigue CB number. These changes, symmetrical to those occurring during fatigue are consistent with the idea that P1 is due to the inhibitory effect of $[P_i]_i$ increase during fatigue whereas P2 could be due to reduction of Ca^{2+}-release and/or reduction of Ca^{2+} -sensitivity of the contractile apparatus at longer times during fatigue.

P5.3

A quantitative method to monitor short and long-term Reactive Oxygen Species (ROS) production kinetics in humans by Electron Paramagnetic Resonance (EPR)

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EPR was recently adopted to develop a physiological relevant method for rapid and noninvasive ROS concentration measurement in fresh human peripheral blood[1]. This innovative approach that showed an increase of ROS levels in short-term high-intensity exercise, is here applied to monitor short and long term kinetics.
Methods: A bench top continuous wave instrument (Bruker) operating in the X-Band region (~ 9 GHz) dealing with very low ROS levels in 50µl samples was used.
Results With respect to basal, ROS levels were found to: **A**) Short term kinetics a) increase after exercise in: healthy young (20±1yr; +7%; $p<0.05$), elder (73±5 yr; +8%; $p<0.05$) and sporadic Amyotrophic Lateral Sclerosis (sALS) patients (57±11yr; +7-19% range); b) decrease after 1 h antioxidant (R(+) Thioctic acid) administration in: healthy subjects (49±5yr; -7.5 % $p<0.05$); patients (type II Diabetic Neuropathies) (65±3yr; -7% $p<0.05$; **B**) Long term kinetics: a) increase in sALS (6 months +10% $p<0.05$) b) decrease in training (32±5yr; -20%; $p<0.001$) subjects and 1 month antioxidant administration in healthy (-8%; $p<0.05$) and diabetic neuropathies (-8%; $p<0.05$). The results were confirmed by a positive statistical correlation with traditional oxidative stress damagemarkers: Protein Carbonyls (PC) and Thiobarbituric acid reactive substances (TBARS).
Conclusions: The method is suitable to quantitatively aestimate oxidative stress kinetic response.

[1.] *Mrakic-Sposta S. et al, Oxid Med Cell Longev. 2012;2012:973927.*

P5.4

Excitability of primary motor cortex in karate athletes: a transcranial magnetic stimulation study

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Aims. Neural efficiency was shown by EEG in karate athletes performing voluntary movements. The aim of this study was to test whether these athletes are characterized by neural efficiency of primary motor cortex excitability.
Methods. Ten right-handed male karate athletes (24.1±5.4 years) and 10 matched non-athletes (27.2±5.1 years) were recruited. EMG motor evoked potentials (MEPs) over the first dorsal interosseus muscle were elicited by transcranial magnetic stimulation delivered over the left primary motor cortex. Motor threshold (MT) was calculated as the highest stimulus intensity that did not produce MEPs in at least 5 to 10 trials in the resting target muscle. MEP latency, peak-to-peak amplitude and area were acquired and compared by the student's t-test and the Pearson's r was used to examine the relationship between variables.
Results. Compared to non-athletes, karate athletes showed lower MT (56.1±4.6 vs 66.0±4.8% of max stimulator output; $p< 0.01$), shorter MEP peak latency (21.8±1.3 ms vs 24.9±1.0 ms; $p< 0.01$) and higher MEP peak-to-peak amplitude and area (120.0±10.0 mV vs 50.0±10.0; $p< 0.05$; 0.7±0.8 mV vs 0.3±0.1 mV; $p<0.05$). Overall correlation between MT and MEP latency was significant ($r=0.5$; $p< 0.05$).
Conclusions. Changes in MT values and MEPs variables may reflect both inhibitory and facilitatory interneuronal circuits, thus sustaining the hypothesis that karate athletes are characterized by a neural efficiency of the primary motor cortex excitability.

P5.5

Blood lactate levels and visual evoked potentials

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We observed, on young athletes, that high blood lactate levels are associated with an enhancement of primary motor cortex excitability. However, it has recently been observed that an increase of blood lactate is not associated with significant changes of brainstem as well as spinal cord excitability. There is no information, to our knowledge, about the possible effects of blood lactate increases, as those evoked by a exhaustive exercise, on excitability other part of the brain. Therefore, in the present study we compare, in young male and female athletes, the effects of high blood lactate levels, induced by performing an exhausting exercise, with the amplitude and latency of the N75, P100, and N135 components of the visual evoked potentials (VEP) waveforms. Subjects were 12 athletes, 6 women and 6 men, aging between 22 and 42 years (mean age 31.9 ± 6.1). Blood lactate levels as well as PEV were measured before, at the end as well as 10 and 20 minutes after the conclusion of the exercise. We observed that blood lactate levels increased from 1.3 mmol/l (± 0.30 SD) before the exercise, to 12.9 mmol/l (± 2.54 SD) at its end and returned to pre-exercise values within 20 min. Amplitude of the N75, P100, and N135 components of PEV did not show significant changes. On the contrary, latency of P100 was significantly reduced ($P < 0.01$) at the end of exercise, while latency of N135 resulted significantly ($P < 0.01$) increased 10 min after the exercise's conclusion.

P5.6

Temperature dependence of myosin filament structure in muscle and in skinned fibres from mammals

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X-ray diffraction patterns were collected from bundles of relaxed skinned fibres from rabbit psoas and from whole intact EDL muscle of mouse at rest at different temperatures (range 10-35 °C). In relaxed skinned fibres at 10°C the M3 reflection from the axial repeat of the myosin heads had a major peak at 14.54 nm (LA peak) and a minor peak at 14.35 nm (HA peak). The intensity of the LA peak reduced with increasing temperature, so that at 35°C the HA peak was dominant as in resting intact EDL muscle at the same temperature. Lowering the temperature in the intact muscle preparation induced a decrease in the total intensity of M3 and an increase in the intensity of LA peak at the expenses of the HA peak. In both preparations lowering the temperature was associated with a decrease in the intensity of the so-called *forbidden* meridional reflections (M2, M4, M5) and of M6 reflection, and an increase in the equatorial reflections intensity ratio. Osmotic compression of the filament lattice in skinned fibres to recover the lattice spacing of intact muscle led to a recovery of the intensity of the meridional reflections and induced a change in the fine structure of M3 reflection similar to that observed on increasing temperature. These effects constrain structural models for the OFF state of the thick filament and its changes upon muscle activation. Supported by FIRB-Futuro in Ricerca and ECRF-Firenze (Italy), MRC (UK).

P5.7

Motor activity and muscle re-innervation: the influence of different patterns of exercise

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The recovery of skeletal muscle innervation after nerve injury remains poorly understood. Recently we found that an intermittent, mid-intensity treadmill activity modulates muscle BDNF inducing axon sprouting and faster muscle re-innervation. In order to understand if different types of motor activity may result in different modulation of muscle re-innervation, we set out to study the effects of different chronic treadmill running protocols or freewheel activity on nerve-lesioned adult rats. After nerve crush, muscle re-innervation and BDNF expression were evaluated using intracellular recordings, tension recordings and western blotting respectively. Surprisingly, a significant increase of multiple muscle innervation was found only with an intermittent, mid-intensity protocol of running compared to sedentary controls. Moreover, a similar tendency was also observed in rats of freewheel activity group, but only in rats voluntary running with high intensity rush. An increased muscle expression of BDNF was found in animals of all running groups. These preliminary results suggest that the re-innervation of skeletal muscle after nerve injury may vary according to the type of motor activity applied. We conclude that an appropriated motor activity might be an important tool in rehabilitation programs after a nerve injury.

P5.8

"Noisy"-patterned extracellular electrical stimuli facilitate muscle cell contractions *in vitro*

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Physical activity is a benefit which improves the quality of life not only in young and healthy people, but also in patients affected by muscle weakness due to ageing, prolonged debilitating illness or traumatic incidents. Electrical stimulation of skeletal muscle cells could mimic *in vitro*, the beneficial effects of physical training, by inducing favorable metabolic changes, fiber-type switches as well as new protein expression. Two different patterns of electrical stimulation were tested in myotubes in culture. Extracellular electrical stimulations were performed via a platinum electrode placed in the field where changes in membrane potential detected by perforated patch recordings could be observed occurring simultaneously with mechanical cell contractions. One minute of "noisy"-patterned electrical stimulation sampled from human quadriceps muscles, when applied to the myotubes, consistently induced action potential activity and cell contractions, whereas the same grade of stimulation, applied as tetanic stimuli at 45 Hz, was not able to induce contractions, unless higher intensity of stimulations were performed. In conclusion, we found that a more physiological "noisy" waveform pattern of electrical stimulation of muscle cells *in vitro* was more efficient in inducing repetitive cell firing and contractions. This information could be useful in the future design of electrical devices to stimulate the rehabilitation/recovery of weakened or injured muscles in human patients.

Design and layout

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