71st SIF National Congress

The Italian Society of Physiology

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Programme & Abstracts

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Programme

Tuesday, 7 September

13:30-14:00 **Welcome messages** [Hall A]

Opening Lecture [Hall A]

14:00-15:00 **José López Barneo** *(University of Seville, Spain)*
Acute oxygen sensing and regulation of breathing

Symposium 1: Cognitive and visuomotor functions in human and non-human primates [Hall A]

Chair: Rossella Breveglieri (Bologna)

15:00-15:20 **Rossella Breveglieri** *(University of Bologna, Italy)*
Visuomotor functions of the medial posterior parietal cortex of monkeys and humans

15:20-15:40 **Simona Monaco** *(University of Trento, Italy)*
Decoding action intention with and without visual information from the activity pattern in the human early visual cortex

15:40-16:00 **Celia Foster** *(Bielefeld University, Germany)*
An expansion of the macaque ventral intraparietal area in the human brain

15:00-16:20 **David Pascucci** *(École Polytechnique Fédérale de Lausanne, Switzerland)*
Modeling large-scale dynamic brain networks during perception and cognition

Symposium 2: Astrocytes-mediated brain handling of neurotoxic molecules [Hall B]

Chair: Roberto Piacentini (Rome) and Alexej Verkhratsky (Manchester, UK)

15:00-15:20 **Myriam Catalano** *(Sapienza University of Rome, Italy)*
Kv1.3 activity modulates astrocyte glutamate buffering

15:20-15:40 **Laura Civiero** *(University of Padua, Italy)*
Astrocyte phagocytic activity: mechanisms in health and disease

15:40-16:00 **Roberto Piacentini** *(Università Cattolica del Sacro Cuore, Rome, Italy)*
Glypican 4 is involved in extracellular tau oligomers internalization in astrocytes

16:00-16:20 **Alexej Verkhratsky** *(University of Manchester, UK)*
Astrocyte iron homeostasis: links to astrogliosis and neurodegeneration

Oral Communications: Cell Physiology 1 [Hall C]

Chair: Annarita Di Mise (Bari)

15:00-15:15 **Rosario Amato** *(Pisa)*
Novel insights into beta 3 adrenergic receptor regulation by oxygen levels in the retina: evidence from the model of oxygen-induced retinopathy

15:15-15:30 **Giorgia Bertoli** *(Milan)*
Electrophysiological characterization of the compound heterozygosity (K1578N-G1866fs) of the cardiac sodium channel (Nav1.5) identified in a young patient affected by sinus node dysfunction

15:30-15:45 **Giorgia Chinigò** *(Turin)*
TRPM8-Rap1A interaction inhibits prostate cancer cell adhesion and migration

15:45-16:00 **Annarita Di Mise** *(Bari)*
Pre-clinical evaluation of dual targeting of the G protein-coupled receptors, CaSR and V2R, for treating Autosomal Dominant Polycystic Kidney Disease (ADPKD)

16:00-16:15 **Marco Fiocchetti** *(Rome)*
Physiological outcomes of a novel estrogen-induced cell signaling pathway

Oral Communications: Neurobiology and Neurophysiology 1 [Hall D]

Chair: Giovanni Mirabella (Brescia)

15:00-15:15 **Francesca Ginatempo** *(Sassari)*
Cerebellar learning is modulated by the view of faces expressing sadness

15:15-15:30 **Anita Monteverdi** *(Pavia)*
Integration of cerebro-cerebellar loops improves virtual brain models of neurodegenerative diseases

15:30-15:45 **Ileana Montagna** *(Pavia)*
Microendoscopic calcium imaging in the cerebellum of freely-moving mice

15:45-16:00 **Giovanni Mirabella** *(Brescia)*
Facial emotional stimuli affect movement preparation, execution and inhibition only when they are task-relevant

16:00-16:15 **Walter Gulisano** *(Catania)*
The Lightmouse project: a complete open-source behavioral system from hardware to AI-based analysis software to study cognition in rodents

16:20-16:30 ***Break***

Symposium 3: Sleep as a phenomenon of the integral organism: recent advances on an old theme [Hall A]

Chair: Alessandro Silvani (Bologna)

16:30-16:50 **Simone Sarasso** *(University of Milan, Italy)*
Sleep-like cortical reactivity during wakefulness following brain injury

16:50-17:10 **Ugo Faraguna** *(University of Pisa, Italy)*
Tracking sleep physiology in the age of wearable technologies

17:10-17:30 **Alessandro Silvani** *(University of Bologna, Italy)*
Recent advances in cardiovascular and motor control during sleep: a translational perspective

17:30-17:50 **Dragana Rogulja** *(Harvard Medical School, USA)*
Sleep and the gut

Symposium 4: What's new on VO2 kinetics? Mechanistic insight and practical implications for exercise testing and prescription [Hall B]

Chair: Silvia Pogliaghi (Verona)

16:30-16:50 **Silvia Pogliaghi** *(University of Verona, Italy)*
VO2 slow component: what's new?

16:50-17:10 **Alessandro L. Colosio** *(Ghent University, Belgium)*
Bioenergetics of the VO2 slow component between exercise intensity domains

17:10-17:30 **Øyvind Nøstdahl Gløersen** *(Norvegian School of Sport Science, Oslo, Norway)*
Modelling VO2 on-kinetics: is it time for a critical revisitation of the traditional 3-phase model?

17:30-17:50 **Lucrezia Zuccarelli** *(University of Udine, Italy)*
Comparison between slow components of heart rate and VO2 kinetics: functional significance and practical implications for exercise administration

Oral Communications: Cell Physiology 2 [Hall C]

Chair: Michele Maffia (Lecce)

16:30-16:45 **Marilina Florio** *(Bari)*
Heterologous production and functional reconstitution of a human aquaglyceroporin of broad selectivity (AQP9)

16:45-17:00 **Federica Giannetti** *(Milan)*
hiPS-cardiomyocytes for studying a specific mutation in PITX2 gene and its role atrial fibrillation insurgence

17:00-17:15 **Michele Maffia** *(Lecce)*
Copper dyshomeostasis in synucleinopathies: a potential role for PLK2 kinase as a counter for a-synuclein aggregation and oxidative stress

17:15-17:30 **Simona Martinotti** *(Alessandria)*
Endothelial integrity recovery: a honey, H2O2 and calcium story

17:30-17:45 **Valentina Melfi** *(Milan)*
The neuroactive steroid ALLO mediates the dualistic role of PKCε in Schwann cell and peripheral sensory neurons

17:45-18:00 **Vincenzo Migliaccio** *(Salerno)*
Beneficial effect of hydroxytyrosol against palmitate-induced lipotoxicity in HepG2 cells: a possible role for mitochondrial fusion and fission balance

Oral Communications: Neurobiology and Neurophysiology 2 [Hall D]

Chair: Fabrizia Cesca (Trieste)

16:30-16:45 **Beatrice Badone** *(Ballerup, Denmark)*
High throughput screening using QPatch II and Qube 384 systems: electrophysiological evaluation of primary neurons, glial cells and hiPSC-derived motor neurons

16:45-17:00 **Veronica Bonalume** *(Milan)*
Axonal GABAA receptor stabilises excitability in unmyelinated nociceptor axons secondary to NKCC1 shift in ECl-

17:00-17:15 **Barbara Barile** *(Bari)*
Impact of AQP4 supramolecular organization in migrating reactive astrocytes

17:15-17:30 **Fabrizia Cesca** *(Trieste)*
Kidins220/ARMS mediates astrocyte developmental switch in BDNF sensitivity, calcium signaling and neuron-astrocyte communication

17:30-17:45 **Elisabetta Catalani** *(Viterbo)*
The pathophysiology of retina neurons through the eye of the fruit fly *Drosophila melanogaster*

17:45-18:00 **Ilaria Piano** *(Pisa)*
MicroRNA-155 is a target to slowing down retinal neuron degeneration in a Retinitis Pigmentosa mice model

17:50-18:15 ***Break***

pH Lecture [Hall A]

18:20 **Lamberto Maffei** *(Pisa)*
Matrimonio tra arte e cervello; officiante il cervello

Wednesday, 8 September

9:00-10:30 **Poster Session 1: Cell Physiology**

10:30-11:00 ***Break***

11:00-12:30 **Poster Session 2: Metabolism, Nutrition and System Physiology**

Herlitzka Lecture [Hall A]

14:00-15:00 **Matteo Carandini** *(UCL, London, UK)*
Recording from a myriad neurons

Symposium 5: An intracranial insight on sensori-motor awareness [Hall A]

Andrea Pigorini and Luca Fornia (Milan)

15:00-15:20 **Gabriele Arnulfo** *(University of Genoa, Italy)*
Mapping human brain network(s) using sEEG: challenges and breakthroughs

15:20-15:40 **Maria Del Vecchio** *(Consiglio Nazionale delle Ricerche, Parma, Italy)*
Tonic somatosensory responses as the neural fingerprint of tactile awareness

15:40-16:00 **Andrea Pigorini** *(University of Milan, Italy)*
Loss of differentiation and complexity in the sleeping human brain: a multi-scale analysis

16:00-16:20 **Luca Fornia** *(University of Milan, Italy)*
Direct electrical stimulation of the premotor cortex shuts down awareness of voluntary actions

Symposium 6: Nutrition in pregnancy: effects on the physiology of maternal uterine circulation and feto-placental unit development [Hall B]

Maurizio Mandalà (Cosenza)

15:00-15:20 **Elizabeth Cottrell** *(Univeristy of Manchester, UK)*
Therapeutic potential of beetroot juice in pregnancy

15:20-15:40 **Teresa Tropea** *(Univeristy of Manchester, UK)*
Beneficial effects of grape seed extract polyphenols in pregnancy

15:40-16:00 **Maurizio Mandalà** *(Calabria University, Italy)*
Effects of Bisphenol A on maternal uterine vasculature and on feto-placental development

16:00-16:20 **Francesca Ietta** *(University of Siena, Italy)*
Metabolic heart adaptation in fetal rats exposed to Bisphenol A

Oral Communications: Cell Physiology 3 [Hall C]

Giulio A Sancini (Milan)

15:00-15:15 **Serena Milano** *(Bari)*
β3-adrenoreceptor participates in the sympathetic regulation of the renal acid-base homeostasis

15:15-15:30 **Sharon Negri** *(Pavia)*
Optical stimulation of endothelial colony forming cells plated on light-sensitive conjugated polymers induces a TRPV1-mediated increase in intracellular Ca2+ concentration

15:30-15:45 **Sofia Passaponti** *(Siena)*
Placenta-brain axis: focus on astrocytes

15:45-16:00 **Giulio A Sancini** *(Milan)*
Considerations around the SARS-CoV-2 spike protein, airborne particulate matter and SARS-CoV-2 brain infection

16:00-16:15 **Rocco Zerlotti** *(Munich, Germany)*
Solid supported membrane-based electrophysiology (SSME) meets SGLT1 and GAT1

Oral Communications: Metabolism, Nutrition and System Physiology [Hall D]

Assunta Lombardi (Naples)

15:00-15:15 **Alex Buoite Stella** *(Trieste)*
Climate change and heat illness risk: a matter for physiologists

15:15-15:30 **Luisa Cigliano** *(Naples)*
Effect of short-term fructose-rich diet on brain: what is reversible and what persists after sugar removal from the diet?

15:30-15:45 **Daniele La Russa** *(Arcavacata di Rende, CS)*
Caloric restriction mitigates kidney fibrosis and apoptosis in an aged and obese rat model

15:45-16:00 **Rita Polito** *(Foggia)*
Effects of very low calorie ketogenic diet on autonomic nervous system activity

16:00-16:15 **Assunta Lombardi** *(Naples)*
Ablation of UCP3 affects visceral white adipose tissue functionality

16:20-16:30 ***Break***

Symposium 7: Passive exercise and vascular function: physiological mechanisms and translational insight [Hall A]

Fabio Esposito (Milan) and Richardson Russel (Salt Lake City, UT, USA)

16:30-16:50 **Emiliano Cè** *(University of Milan, Italy)*
Long-term passive static stretching and vascular function

16:50-17:10 **Joel Trinity** *(University of Utah, USA)*
Passive leg movement and the endothelium: looking beyond NO

17:10-17:30 **Massimo Venturelli** *(University of Verona, Italy)*
Passive leg movement-based training: the vascular impact

17:30-17:50 **Walter Wray** *(University of Utah, USA)*
Vascular function and blood flow regulation in patients with heart failure

Symposium 8: New vistas on cerebellar circuit dynamics [Hall B]

Egidio D'Angelo (Pavia)

16:30-16:50 **Lisa Mapelli & Claudia Casellato** *(University of Pavia, Italy)*
Single neurons and circuits of the cerebellum

16:50-17:10 **Jil Mona Meier** *(Charité Universitätsmedizin Berlin, Germany)*
Cerebellar dynamics in virtual brain simulators

17:10-17:30 **Fulvia Palesi** *(University of Pavia, Italy)*
Exploring the cerebellum in humans through MRI recordings and virtual brain modelling

17:30-17:50 **Silvia Marchese** *(University of Milan, Italy)*
Virtual brain modelling in cerebellar ataxic patients

Oral Communications: Neurobiology and Neurophysiology 3 [Hall C]

Marco Luppi (Bologna)

16:30-16:45 **Carmen Murano** *(Milan)*
Functional characterization of a novel *HCN2* variant associated with progressive epileptic encephalopathy in neonatal rat neurons

16:45-17:00 **Marlene E Pfeffer** *(Genoa)*
Engineering of azobenzene-derived membrane-targeted photoswitches for light-driven modulation of neuronal activity

17:00-17:15 **Ingrid Reverte** *(Rome)*
Effect of social choice-induced voluntary abstinence on incubation of methamphetamine craving and AMPA receptor expression in nucleus accumbens core

17:15-17:30 **Marco Luppi** *(Bologna)*
Synthetic torpor stimulates a latent physiological neuroprotective process able to cope with the brain accumulation of hyperphosphorylated tau protein

17:30-17:45 **Maria Rosaria Tropea** *(Catania)*
A failure of Amyloid-β physiological function due to deletions of α7 nicotinic acetylcholine receptors triggers an Alzheimer's disease-like pathology

17:45-18:00 **Chiara Piantoni** *(Hannover, Germany)*
Age-related changes in cardiac autonomic modulation and heart rate variability in mice

Oral Communications: Metabolism and Neurobiology [Hall D]

Valentina Pallottini (Rome)

16:30-16:45 **Valentina Pallottini** *(Rome)*
Maternal exposure to very low dose of BPA induces alteration of mevalonate pathway in the liver and brain of rat fetuses

16:45-17:00 **Eleonora Solari** *(Varese)*
Involvement of TRPVs channels in the response to fluid osmolarity by rat diaphragmatic lymphatic vessels

17:00-17:15 **Maria Grazia Zizzo** *(Palermo)*
Does Angiotensin II contractile response undergo to age-related changes in rat jejunum?

17:15-17:30 **Mariangela Centrone** *(Bari)*
Vasopressin downregulates the AQP3 function via V1aR in human colon HCT8 cells

17:30-17:45 **Giuseppina D'Alessandro** *(Rome)*
Environmental signals modify gut microbiome and metabolome enhancing neural plasticity through short-chain fatty acids

17:45-18:00 **Tiziana Romanazzi** *(Varese)*
The interaction of bile acids with dopamine transporter heterologously expressed in *Xenopus laevis* oocytes

17:50-18:15 ***Break***

Plenary Lecture [Hall A]

2019 Nobel Laureate in Physiology or Medicine

18:20 **Gregg L. Semenza** *(Johns Hopkins University, Baltimore, MD, USA)*
Hypoxia-Inducible Factors in Physiology and Medicine
Sponsored by Umana Medical a brand of GPI, Malta

Thursday, 9 September

9:00-10:30 **Poster Session 3: Neurophysiology and Neurobiology**

10:30-11:00 ***Break***

11:00-12:30 **Poster Session 4: Physiology of Motor System and Exercise**

Ruzzier Lecture [Hall A]

14:00-15:00 **Marco Linari** *(University of Florence, Italy)*
The role of thick filament mechanosensing in the Starling law of the heart

Symposium 9: New insights on neuromuscular control through advanced High Density EMG analysis [Hall A]

Daniele Borzelli (Messina)

15:00-15:20 **Kohei Watanabe** *(Chukyo University, Toyota, Japan)*
Regional activation within human rectus femoris muscle; physiological/biomechanical background and methodological applications

15:20-15:40 **Daniele Borzelli** *(University of Messina, Italy)*
CNS exploits separate interneuronal pathways to control force and cocontraction

15:40-16:00 **Taian Martins Vieira** *(Politecnico di Torino, Italy)*
Physiological and technical insights gained into the assessment of muscle function from a combined approach: high-density surface EMG and electrical stimulation

16:00-16:20 **Simone Tanzarella** *(IIT, Genoa, Italy)*
Motor neuron synergistic organization in complex hand gestures

Symposium 10: Unconventional pathways and targets in the onset of cardiac arrhythmias [Hall B]

Marcella Rocchetti and Annalisa Bucchi (Milan)

15:00-15:20 **Ji-Dong Fu** *(The Ohio State University, USA)*
MicroRNA modulates cardiac electrophysiology via direct binding to ion channel

15:20-15:40 **Pier Leopoldo Capecchi** *(University of Siena, Italy)*
Autoimmune channelopathies as a novel mechanism in cardiac arrhythmias

15:40-16:00 **Maria Fernandez Velasco** *(Hospital Universitario La Paz, Madrid, Spain)*
Innate immune receptors, key actors in cardiovascular diseases

16:00-16:20 **Ilaria Rivolta** *(University of Milan Bicocca, Italy)*
KNa1.1: a novel player in cardiac electrophysiology

Oral Communications: Motor System and Exercise [Hall C]

Feliciano Protasi (Chieti)

15:00-15:15 **Riccardo Borzuola** *(Rome)*
Neuromuscular electrical stimulation affects spinal excitability but not cortical activity of the somatosensory cortex

15:15-15:30 **Roberto Panichi** *(Perugia)*
Motor control of upper limb movement: functional significance of sensory overactivation and visual-proprioceptive interaction

15:30-15:45 **Veronica Farinelli** *(Milan)*
A novel viewpoint on anticipatory postural adjustments during gait initiation

15:45-16:00 **Feliciano Protasi** *(Chieti)*
Calcium Entry Units: focus on the alternative door for calcium ions in skeletal muscle

16:00-16:15 **Anna Taboni** *(Geneva, Switzerland)*
Baroreflex and operating point dynamics at exercise onset in hypoxia

16:20-16:25 **Concluding Remarks** [Hall A]

16:30-18:30 **SIF General Assembly** [Hall A]

Speakers' Abstracts

In chronological order of presentation
(presenting authors are shown underlined)

Visuomotor functions of the medial posterior parietal cortex of monkeys and humans

Rossella Breveglieri, M. Filippini, M. De Vitis, P. Fattori
Dept Biomedical and Neuromotor Sciences, University of Bologna
Funded by H2020-EIC-FETPROACT-2019 n.951910-MAIA

Visuomotor coordination is essential to integrate visual information to generate and control the correct motor output, that is required for the act of prehension. The medial posterior parietal cortex (mPPC) is a key region of humans and monkeys' brain for coordinating visuomotor behavior in a rapid, accurate, and flexible goal-driven manner. Among mPPC different subregions, an area active during manual interactions is V6A, which is located in the anterior bank of parieto-occipital sulcus, within monkey Brodmann's area 7. Being an association area, V6A receives different sensory input, namely visual and somatosensory, and uses these signals to estimate the state of the arm in order to perform correct visuomotor actions. Moreover, V6A contains cells modulated by reaching and grasping movements. Recently, monkey V6A has been shown to encode depth and direction of gaze position and reaching, and this is very useful for acting in the three-dimensional space. Also human V6A (hV6A) is located in the posterior part of Brodmann's area 7 and shares many functions with monkey V6A, like the activation during reaching and grasping in fMRI experiments. Recently in our lab, the causal role of hV6A was investigated using transcranial magnetic stimulation: impairment in the encoding of depth of reaching during reach planning was observed after hV6A stimulation. All these experiments demonstrated that monkey and human V6A subserve crucial functions to interact with the external world.

Decoding action intention with and without visual information from the activity pattern in the human early visual cortex

Simona Monaco, G. Malfatti, L. Pizzato, L.Turella
CIMeC - Center for Mind/Brain Sciences, University of Trento, Italy

The human early visual cortex (EVC) comprises feedback projections from higher-level cortical areas that profoundly affect perception. Yet, little is known about how the preparation of actions modulates the activity in the EVC. Here we used fMRI while participants (N=16) performed actions with the right dominant hand towards a 3D-real-object. We manipulated the availability of visual information (Vision or No Vision) and action type (Grasp or Open hand). With multivoxel pattern analyses, we examined whether the activity pattern in the Foveal confluence, corresponding to the retinotopic location of the object in the Vision condition, could be used to decode action intentions (Grasp vs. Open hand) during the planning phase preceding the action. Our results show successful decoding for the dissociation between action types in Vision and No Vision conditions in the Foveal confluence as well as in V1, V2, and V3 areas corresponding to peripheral vision. Interestingly, the activity patterns in Vision condition could be used to successfully dissociate action intentions in No Vision condition, and vice versa. These findings indicate that action planning modulates the activity patterns in widespread regions of the EVC, regardless of object location. Further, action intention can be decoded even in absence of visual information, suggesting predictive coding mechanisms related to sensory-motor and memory information that is recruited regardless of the availability of online visual input

An expansion of the macaque ventral intraparietal area in the human brain

Celia Foster[1], W.-A. Sheng[2], S. Ben Hamed[2], T. Heed[1]
[1]Biopsychology and Cognitive Neuroscience, Faculty of Psychology and Sports Science, Bielefeld University, Bielefeld, Germany
[2]Institut des Sciences Cognitives Marc Jeannerod, UMR 5229, Université de Lyon – CNRS, Bron, France

The macaque ventral intraparietal area (VIP) is involved in a variety of functional processes, including motion processing, multisensory integration, processing of near-head space, defensive sensorimotor behaviour and numerosity coding. Human fMRI studies have attempted to define the location of a putative human homologue of the macaque VIP (pVIP) by investigating which human brain regions respond to subsets of stimuli known to elicit activity in macaque VIP. In this work, we conducted a comprehensive literature review to find all studies that propose a pVIP location and then projected the given pVIP coordinates onto the cortical surface to compare their locations. We found that proposed pVIP coordinates were widely dispersed across a large area of parietal cortex. However, coordinates from three different commonly used methods to functionally localize pVIP showed converging, bilateral clusters within each method, but separated clusters across the different methods, suggesting that these different localization methods target different functional regions. This finding suggests that VIP has diverged into three separate regions in the human brain, which is compatible with the known expansion of human posterior parietal cortex and evidence of anatomical and functional variation within macaque VIP. However, there are still many aspects of a macaque human comparison that are still unclear and will need to be addressed to fully clarify VIP homology between the two species.

Modeling large-scale dynamic brain networks during perception and cognition

David Pascucci
Brain Mind Institute, École Polytechnique Fédérale de Lausanne (EPFL), Switzerland

In the last decade, the rising field of network neuroscience has emphasized the need for advanced functional connectivity measures. A major goal is to understand the dynamics of directed and large-scale neuronal interactions that underlie perception, cognition, and action. Modeling network interactions that evolve at the sub-second timescale of brain functions, however, remains a major ongoing challenge. Here, I will describe an endeavor to characterize fast dynamics in functional brain networks during evoked brain activity. I will present an extension of classical linear adaptive filters for modeling event-related changes in directed connectivity patterns, using electroencephalography and source imaging data. Within this modeling framework, I will then evaluate the advantages of combining structural and functional connectivity, under a multimodal imaging scheme. After introducing the methods, I will review recent results of their application in the field of human perception and attention, focusing on how accurate models of time-varying brain connectivity could yield new fundamental insights into the dynamic and frequency-specific computations behind cognition and behavior.

Kv1.3 activity modulates astrocyte glutamate buffering.

A. Grimaldi[1], G. D'Alessandro[2,3], M. A. Di Castro[3], C. Lauro[3], V. Singh[4], F. Pagani[1], L. Sforna[5], F. Grassi[3], S. Di Angelantonio[1,3], L. Catacuzzeno[6], H. Wulff[4], C. Limatola[2,3], Myriam Catalano[3]
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[3]Department of Physiology and Pharmacology, Sapienza University, Rome, Italy
[4]Department of Pharmacology, University of California, Davis, USA
[5]Department of Experimental Medicine, Section of Physiology and Biochemistry, University of Perugia, Perugia, Italy
[6]Department of Chemistry Biology and Biotechnology, University of Perugia, Perugia, Italy

Glial cells actively maintain the homeostasis of brain parenchyma, regulating neuronal excitability and preserving the physiological composition of the extracellular milieu. Under pathological conditions, some functions of glial cells could be compromised, exacerbating the neurotoxic processes. We investigated if the homeostatic activities of astrocytes could be modulated by the voltage-gated K^+ channel Kv1.3. To this end we used *in vitro* and *in vivo* systems to model cell-to-cell interactions in tumoral conditions, using a specific inhibitor of Kv1.3 channels, 5-(4-phenoxybutoxy) psoralen (PAP-1). We demonstrated that PAP-1 increases astrocytic glutamate uptake, reducing glioma-induced neurotoxicity. We also found that Kv1.3 activity is required for blood brain barrier integrity. The crucial role of Kv1.3 channels as modulators of astrocyte activity was confirmed in a mouse model of glioma, where PAP-1 treatment reduces tumor volume only in the presence of active glutamate transporters GLT-1. All these findings point to Kv1.3 channels as potential targets to re-instruct astrocytic cells toward their homeostatic functions, in the context of brain tumors.

Astrocyte phagocytic activity: mechanisms in health and disease

Laura Civiero
Dept Biology, Univ. of Padua, Padua, Italy

The maintenance of a healthy state of the central nervous system depends on the immediate removal of toxic, obsolete or unwanted material thus preventing inflammatory events. Under certain conditions, astrocytes demonstrate phagocytic capability and cooperate with microglia as an ancillary clerance system in the brain. Astrocyte processes are in close association with synapses and contribute to synaptic health at several levels. Noteworthy, astrocytes are efficient sensors of synaptic dysfunction or degeneration.
Indeed, they intervene by eliminating neuronal terminals, engulfing debris and internalizing neuronal-released aggregated proteins. However, the molecular machinery recruited for the recognition of specific targets is only in part clarified. In my talk, I will first introduce a novel molecular pathway used by astrocytes to clear alpha-synuclein aggregates; then, I will present a cell-based high-throughput approach to identify new players in astrocyte-mediated synaptic phagocytosis.

Glypican 4 is involved in extracellular tau oligomers internalization in astrocytes

Roberto Piacentini[1,2]
[1]Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy
[2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Several studies reported the detrimental effects of extracellular tau oligomers (ex-oTau) on glutamate-dependent synaptic transmission and plasticity (1). Astrocytes efficiently internalize ex-oTau and contribute to their clearance, but intracellular oTau accumulation leads to alteration of Ca^{2+}-dependent gliotransmitter release impinging on synaptic transmission (2).
Heparan sulfate proteoglycans (HSPGs) are membrane glycoproteins acting as receptors for several extracellular ligands involved in tau internalization in cells (3). Indeed, astrocytes express high levels of HSPGs (4). Glypicans (GPCs) are a subfamily of HSPGs, and the astrocyte-specific GPC4 regulates the functional development of glutamatergic synapses (5), that are key targets of oTau.
Here we report that occluding GPC4-oTau interaction by a specific anti-GPC4 antibody applied 24 hours prior to 200 nM ex-oTau treatment significantly reduces oTau internalization in astrocytes and prevents oTau-induced intracellular Ca^{2+} signaling deregulation. Anti-GPC4 also spares from the synaptotoxic action of ex-oTau both *in vitro* and *ex vivo*. Similar results were also obtained in experimental models in which GPC4 expression was reduced or lacking.
Our data indicate that GPC4 is a mediator of tau internalization in cells and a potential therapeutic target for tauopathies.

References
Puzzo D et al., eLife 2017
Piacentini R et al., Glia 2017
Holmes BB et al., PNAS 2013
Li Puma DD et al., Glia 2021
Allen N et al., Nature 2012

Astrocyte iron homeostasis: links to astrogliosis and neurodegeneration

Alexei Verkhratsky
Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK

Iron contributes to numerous cellular and biochemical processes and acts as a co-factor in various molecular cascades in the nervous tissue including the synthesis and metabolism of several brain-specific enzymes and neurotransmitters. Plasmalemmal divalent metal ion transporter 1 (DMT1) is responsible for cellular uptake of ferrous (Fe^{2+}), whereas transferrin receptors (TFR) carry transferrin (TF)-bound ferric (Fe^{3+}). Uptake of Fe^{2+} by DMT1 inhibits astroglial Na^+-K^+-ATPase, which leads to elevation in cytoplasmic Na^+ concentration, thus reversing Na^+/Ca^{2+} exchanger and thereby generating Ca^{2+} influx. Uptake of Fe^{3+} by TF-TFR stimulates phospholipase C to produce inositol 1,4,5-trisphosphate ($InsP_3$), thus triggering $InsP_3$ receptor-mediated Ca^{2+} release from endoplasmic reticulum. Excess of iron in the brain as well as aberrant regulation of iron homeostasis by neuroglia contributes to the pathogenesis of neurodegenerative and neuropsychiatric diseases. Analysis of *anamnesis morbi* of patients receiving iron implants revealed higher incidence of Parkinson's diseases and ischemic stroke. Injection of iron dextran to the brain of mice selectively decreased presence of DMT1 in neurones while increasing its expression in astrocytes and microglia and triggered reactive astrogliosis and microgliosis. Excess iron from surgical implants thus can affect neural cells and may be regarded as a risk factor for neurodegeneration.

Novel insights into beta 3 adrenergic receptor regulation by oxygen levels in the retina: evidence from the model of oxygen-induced retinopathy

Rosario Amato[1], F. Pisani[2], E. Laudadio[3], M. Lucchesi[1], S. Marracci[1], M. Cammalleri[1], L. Filippi[4], R. Galeazzi[5], M. Svelto[2,6,7], P. Bagnoli[1], M. Dal Monte[1]
[1]Department of Biology, University of Pisa, Pisa, Italy
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Oxygen-sensing mechanisms controlled by the hypoxia-inducible factor-1 (HIF-1) are a primary drive for angiogenic processes triggered by reduced oxygen tension. In this context, β-adrenoceptors (BARs) have been found involved in the hypoxia-dependent growth of new blood vessels. In particular, BAR3 levels seem to be strictly linked with oxygen tension, but its connection with oxygen-sensing factors is still unclear. In this study, we tested whether BAR3 expression is directly regulated by HIF-1 during retinal vascular development and in the context of oxygen-induced retinopathy (OIR).
We showed that BAR3 expression inversely correlates with oxygen tension during retinal development and in the OIR model. BAR3 expression was related with HIF-1 levels but was altered after HIF-1 stabilization. From a reanalysis of the BAR3 gene, we found 6 new putative HIF-binding sites (HBS), one of which met the features of a likely enhancer. The possibility of the HIF-1 bound to this HBS was confirmed by molecular docking simulations and demonstrated by ChIP-qPCR, displaying the correlation of the HBS enrichment with the oxygen variation in the OIR model. first evidence of HIF-1 regulation of BAR3 expression in the retina, paving the way to the characterization of the BAR3 role in oxygen-dependent adaptation processes.

Electrophysiological characterization of the compound heterozygosity (K1578N-G1866fs) of the cardiac sodium channel ($Na_v1.5$) identified in a young patient affected by sinus node dysfunction

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Alterations of the *Scn5a* gene (NaV1.5 channel) are often related to severe forms of cardiac arrhythmias. We studied a compound heterozygosity (K1578N/G1866fs) identified in a child affected by sinus node dysfunction implanted with a pacemaker. Mutant NaV1.5 currents were investigated in HEK293 cells by means of patch-clamp experiments to identify the causative role of the mutations. Neonatal (Neo) and adult (Ad) channels are known to be differently regulated by splicing events with the former expressing the exon 6A and the latter the exon 6B. WT Neo and Ad channels displayed similar conductance, but both the Neo activation and inactivation curves were positively shifted (~10mV and 8 mV, respectively). Compound expression of the mutant condition (K1578N/G1866fs) yielded a dramatic reduction of the current both in the Neo (6A) and in the Ad (6B) channel background (~55% reduction). Single mutations were also expressed in the Ad background and no significant differences were observed when compared to the WT condition. In conclusion, our data reveal a loss-of-function nature of the compound heterozygosity which may partly explain the reported sinus dysrhythmias. Since this l-o-f is maintained throughout the developmental switch of Nav.15 splicing variants, we hypothesize that a clinical impact should also be present during adulthood. The finding that single mutants are not different from the Ad/WT channel is compatible with the healthy conditions of the parents.

TRPM8-Rap1A interaction inhibits prostate cancer cell adhesion and migration

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Emerging evidence indicates that TRPM8 channel plays an important role in prostate cancer (PCa) progression, by impairing the motility of these cancer cells. TRPM8 expression in PCa was shown to be regulated by steroid hormones and receptor signaling and indeed, it results down-regulated in metastatic androgen-independent PC3 cells.
Here, we investigate the mechanism through which TRPM8 inhibits PCa motility, showing that it involves a direct protein-protein interaction of the channel with the small GTPase, Rap1A, as evidenced by active Rap1 pull-down assays and live-cell imaging experiments. Moreover, molecular modeling analyses allowed the identification of four putative residues involved in TRPM8-Rap1A interaction. GST-pull-down and co-immunoprecipitation experiments confirmed the importance of residues E207 and Y240 in the sequence of TRPM8 and Y32 in that of Rap1A in the interaction between the two proteins. The functional role of these mutants was further validated by adhesion and migration assays on PCa cells, which showed a less prominent inhibition of cell motility in cells overexpressing TRPM8 E207A Y240A with respect to those overexpressing TRPM8 wt.
Our data indicate that TRPM8 impairs PCa motility by intracellularly trapping Rap1A in its inactive form, thereby preventing its activation at the plasma membrane. The resulting protective role exerted on metastatic PCa provides new insight into the possible use of TRPM8 as a new therapeutic target in PCa treatment.

Pre-clinical evaluation of dual targeting of the G protein-coupled receptors, CaSR and V2R, for treating Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Autosomal Dominant Polycystic Kidney Disease (ADPKD), caused by mutations in *PKD1* or *PKD2* genes, is characterized by development and growth of cysts causing progressive kidney enlargement and failure. Reduced resting cytosolic calcium and increased cAMP levels, associated with the tonic action of vasopressin, are two central biochemical defects in ADPKD. No drug has been shown to be effective to cure ADPKD, the current treatments are only efficacious in slowing cysts growth. Lixivaptan, a novel vasopressin V2 receptor (V2R) antagonist, is expected to have a safer liver profile compared to tolvaptan, the only drug approved to delay PKD progression.
Here we show that co-targeting two GPCRs, the V2R and the Calcium Sensing Receptor (CaSR), using the V2R antagonist lixivaptan in combination with the calcimimetic R-568, reduced cyst progression in two animal models of human PKD, PCK rats and *Pkd1*$^{RC/RC}$ mice, fed ground rodent chow without or with lixivaptan and R-568, alone or in combination. In PCK rats, the combined treatment strongly decreased kidney weight, cyst and fibrosis volumes by 20%, 49% and 73%, respectively, compared to untreated animals. In *Pkd1*$^{RC/RC}$ mice, the same parameters were reduced by 20%, 56% and 69%, respectively.
In both cases the combined treatment appeared nominally more effective than the individual drugs used alone. The potential for synergy between these two existing drugs in the treatment of ADPKD warrants further investigation in clinical settings.

Physiological outcomes of a novel estrogen-induced cell signaling pathway

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17β-estradiol (E2) regulates a myriad of physiological processes in mammal cells, including the subtle balance between cell survival and apoptosis, through its receptors (i.e. ERα and ERβ). Recently, we identified a novel signaling pathway that culminate in the accumulation of the monomeric globin Neuroglobin (NGB). Here, the mechanisms and the role played by NGB as molecular determinant and functional mediator of E2 cellular effects will be reported. Data indicate that context-specific pathways are triggered by E2 depending from the receptor subtype expressed. These pathways, which converge on the NGB accumulation in the cytosol and the mitochondria, are at the root of the anti-apoptotic and anti-oxidant hormone-activated effects devoted to cell survival against stresses of different nature. To complicate further the scenario, our results demonstrate the NGB extracellular release under E2 and/or stressing stimuli where NGB can act as autocrine/paracrine factor able to communicate cell resilience against stress, widening the vision of the cellular response to external stimuli beyond the intracellular context. Altogether, reported results clarify the role of NGB as an E2-inducible and compensatory protein involved in the E2 cytoprotective effects.

Cerebellar learning is modulated by the view of faces expressing sadness

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Recent studies argued the relevance of the cerebellum in emotions' processing. However, its role in the processing of face expressions is still unknown. The present study investigated the effect of face emotional expressions on cerebellar learning processes, using the eyeblink classical conditioning (EBCC) as a model. Visual stimuli composed of faces expressing happy, sad and neutral emotions were used as conditioning stimulus in forty healthy subjects to modulate the EBCC. The effect of these stimuli was also investigated on the blink reflex (BR) and its recovery cycle (BRRC) and on the cerebellar-brain inhibition (CBI). Results revealed that, in comparison with EBCC, the learning component of the reflex was significantly reduced following the passive view of sad faces, while its extinction phase was decreased by the view of both sad and happy faces. BR, BRRC and CBI were not significantly affected. Data provide first evidence that faces emotional expressions are processed by the cerebellum, with no apparent involvement of the brainstem and the cerebello-cortical connection. In particular, the excitability of the cerebellar circuit underlying the learning phase of the EBCC was selectively reduced by the view of sad faces. Differently, the extinction phase was shortened by both happy and sad faces, compared to neutral ones, suggesting that different neural bases underlie the learning and extinction of emotions expressed by faces.

Integration of cerebro-cerebellar loops improves virtual brain models of neurodegenerative diseases

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The Virtual Brain (TVB) is a novel neuroinformatic platform developed to simulate whole-brain dynamics starting from individual structural and functional connectivity. To date, TVB has been used to characterize brain dynamics in healthy and pathological subjects, but cerebellar nodes and their connections have been overlooked. This is at odd with the recent observation that integrating cerebro-cerebellar connections can improve TVB predictive capability in healthy subjects (HC). Moreover, cerebellar impairment has been reported in neurodegenerative diseases, like Alzheimer's disease (AD), Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). In this work we integrated cerebro-cerebellar connections in TVB to assess the cerebellar impact on simulated brain dynamics both in physiological (HC) and neurodegenerative conditions (AD, FTD, ALS). Our findings demonstrated that the integration of cerebro-cerebellar connections improved the predictive power of the model in pathologies. Moreover, the biophysical parameters derived from TVB differed between the clinical phenotypes considered, contributed to explain the variation of neuropsychological scores and provided a unique description of the excitatory/inhibitory balance at the single subject level. Overall, this work supports the cerebellar involvement in neurodegenerative states, opens new perspectives in the use of TVB to explore neurodegenerative mechanisms and lays the basis for future personalized medicine.

Microendoscopic calcium imaging in the cerebellum of freely-moving mice

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Microendoscopic Ca^{2+} imaging through genetically encoded Ca^{2+} indicators combined with viral tools represents a recent cutting-edge approach for monitoring neuronal populations activity in specific brain regions. In this work, we show a pioneering study of Ca^{2+} imaging recordings of cerebellar activity using miniaturized head-mounted microscopes in freely-moving mice performing a motor-task on a custom-built treadmill. Although the cerebellar role in motor processing is undoubted, the spatiotemporal dynamics and interplay among neuronal subtypes during movement are still largely unknown, mostly for technical limitations due to freely-moving conditions. Neuronal Ca^{2+} dynamics in the cerebellar cortex during locomotion were tracked over several days, allowing to investigate movement-dependent patterns of activity. The accurate analysis of neuronal response patterns over time is being used to unravel spatiotemporal dynamics of cerebellar motor integration and elucidate neuronal correlates of behavior. These data, though preliminary, show the impressive potential of this approach in studying cerebellar physiological mechanisms in feely-moving rodents and open new perspectives in the investigation of cerebellar behavioral paradigms addressing sensorimotor integration and associative learning. Importantly, these experiments will be able to provide unique data to be integrated in computational models, thus enabling a multimodal approach to investigate cerebellar functions.

Facial emotional stimuli affect movement preparation, execution and inhibition only when they are task-relevant

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Facial emotional expressions play a crucial role in social interactions. It has been claimed that such stimuli have a special status in that they can bias selective attention, reflexively prioritizing their processing. Nevertheless, experimental evidence has provided mixed results. A key factor explaining such inconsistencies is the task relevance of the stimuli' emotional content. To investigate how emotional expressions affect motor planning, I gave two versions of a Go/No-go task to healthy participants. In the *emotional* version, they had to execute a reach at the presentation of emotional faces (fearful, angry, happy) and withhold it when neutral faces were shown. The same pictures were displayed in the other version, but participants had to act solely according to the actor's gender. In addition, I used the same experimental design to study the influence on inhibitory control. This time, in the *emotional* task, participants had to withhold the movement when emotional faces (fearful, happy) were presented and perform it at the presentation of neutral faces. First, I found that negative expressions increased the reaction times and the percentages of errors for happy expressions. In the second set of experiments, I discovered that fearful expressions improved inhibitory control for happy expressions. Notably, these effects occurred only when facial emotions were task-relevant. These results suggest that emotional stimuli influence behavior when relevant for ongoing goals.

The Lightmouse project: a complete open-source behavioral system from hardware to AI-based analysis software to study cognition in rodents

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Exploration based paradigms represent gold standard techniques in neuroscience to study cognitive function in animal models. However, these methods are heavily influenced by the high variability of experimental settings and analysis processes across laboratories, thus critically affecting results reliability.
To standardize the behavioral system, we first focused on the experimental context. To this end, we designed a customizable behavioral apparatus with the computer aided design software SolidWorks. The apparatus was composed by a frame of aluminum profiles holding an arena of anti-glare acrylic panels with a 3D printed hosting mechanism allowing an easy change of the experimental setting. A CMOS sensor camera and lightning system were fixed on the lid of the frame. Then, to improve and automate the analysis process, we trained a Deep Learning system. We used the DeepLabCut toolbox to extrapolate spatial coordinates of the selected body parts of the animal across the video frames to obtain its precise position in the arena. These coordinates were then computed through an algorithm to determine the time spent in precise places to be used for further analyses.
Our final goal is to create an open access repository for other neurophysiology laboratories to build customized behavioral apparatus and download an already trained Deep Learning based software to optimize the experimental outcomes.

Sleep-like cortical reactivity during wakefulness following brain injury

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The hallmark of physiological sleep is the occurrence of slow waves and off-periods, often referred to in the sleep literature as cortical bistability, whose cellular determinants have been extensively characterized in vitro, in vivo as well as in computo. Here, I will present recent evidence showing the intrusion of a pathological form of sleep-like cortical bistability during wakefulness following focal as well as severe brain injury. Specifically, using a perturbational approach through a combination of transcranial magnetic stimulation and electroencephalography (TMS/EEG), we detected the occurrence of prominent sleep-like TMS-evoked slow waves and off-periods in awake brain-injured patients of various etiologies. These events were associated with a disruption of causal cortical interactions and signal complexity whose spatial extent matched the patient's degree of structural as well as functional impairment (ranging from cognitive/motor symptoms to disorders of consciousness). Overall, these results link potentially reversible neurophysiological events to the network consequences of brain injury and may represent a valid read-out of the state of discrete cortical circuits as well as a potential target for the development of novel therapeutic interventions aimed at fostering functional recovery.

Tracking sleep physiology in the age of wearable technologies

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Measuring and quantifying sleep, both in the basic research field as well as in the clinical practice, requires the application of the gold-standard approach, i.e. the polysomnographic (PSG) recording of one or more nights. Nevertheless, PSG is suboptimal for naturalistic long-term sleep detections. In parallel, the widespread availability of wearable sensors in the consumer market of wearable devices, such as smartbands and smartwatches, offers unprecedented opportunities to collect large-scale sleep-related data. Unfortunately, most consumer devices did not face proper and careful scientific validation versus the gold-standard, neither medical certification. In this presentation, I will propose novel validation and certification data testing procedures, taking advantage of machine-learning methods as a viable approach towards the collection and analysis of accurate sleep-related data from consumer devices.

Recent advances in cardiovascular and motor control during sleep: a translational perspective

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Increases in arterial blood pressure (ABP) during nighttime sleep increase the risk of adverse cardiovascular events. Nighttime ABP is controlled mainly by sleep-related mechanisms, with an important role of sympathetic vasoconstriction. Accordingly, altered nighttime ABP control is common in subjects with chronic insomnia or the restless legs syndrome (RLS), which are highly prevalent diseases that negatively impact on nocturnal sleep. RLS is also associated with frequent leg movements during sleep (LMS). Periodic and aperiodic LMS with different time structures depend on different (dopaminergic vs histaminergic) neurotransmitter mechanisms. Nevertheless, all LMS entail surges in ABP and heart rate that are shaped by the interaction between central autonomic commands and arterial baroreflex control. The central autonomic network that drives sleep-related cardiovascular changes includes the hypothalamic orexin (hypocretin) neurons. Orexin deficiency blunts the nighttime ABP decline in animal models and in patients with narcolepsy type 1 (NT1) and enhances atherosclerosis burden in animal models of NT1 or of chronic sleep fragmentation. Orexin deficiency also entails the loss of sleep atonia in animal models and in patients with NT1, possibly due to modulation of the pontine sublaterodorsal nucleus activity, and is associated with frequent LMS in patients. Cardiovascular and motor control are, thus, tightly coupled during sleep and highly relevant to cardiovascular health.

Sleep and the gut

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It has long been maintained that sleep is all about the brain but we recently showed that this is not true. Although the nervous system is central for generating sleep, many systemic diseases are caused by sleep loss and animals even die if prevented from sleeping. We showed that the organ critically affected by sleep loss is the gut. The death of sleep-deprived animals can be prevented if the gut is kept healthy. Even more recently we showed that the relationship between the gut and sleep goes both ways - signals originating in the gut control sleep depth. I will talk about our efforts to understand the connections between the gut and sleep.

$\dot{V}O_2$ slow component: what's new?

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Mathematical modelling of the $\dot{V}O_2$ response during metabolic transitions (i.e. $\dot{V}O_2$ kinetics) has offered a non-invasive means to probe the physiological mechanisms that regulate/limit O_2 delivery and utilization in humans. The clarification of the relative role of the above factors and their sensitivity to interventions inform effective measures to maintain mobility/increase exercise tolerance and improve health throughout life. We will present some new insights related to the $\dot{V}O_2$ kinetics, with special reference to the so-called slow component of $\dot{V}O_2$, and the underlying physiological determinants.
A $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) develops as a function of time during constant load exercises performed above the heavy-intensity boundary, impairing exercise tolerance. Type I fibers fatigue and/or increased type II fibers recruitment are putative causes of this loss of efficiency. We will present new data on the effect of acute manipulations of maximal muscle strength on the slow component of oxygen consumption. Acute strength manipulations, by altering maximal force and, in turn, the extent of muscle activation at a given exercise intensity, are associated with modifications of intramuscular metabolic (in)stability, of the amplitude of slow component of $\dot{V}O_2$ and ultimately also affect exercise tolerance. The data suggest a possible link between the recruitment of high-threshold motor units at a given exercise intensity, the $\dot{V}O_2$ slow component and exercise tolerance.

Bioenergetics of the $\dot{V}O_2$ slow component between exercise intensity domains

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During heavy and severe constant load exercise, oxygen consumption (VO_2) displays a slow component (VO_{2sc}) typically interpreted as a loss of efficiency of locomotion developing after the first minutes of exercise. In this study, eight active men performed 3 constant load trials of 3, 6 and 9 min in the moderate, heavy and severe domains (*i.e.* respectively below, between, and above the two ventilatory thresholds) to quantify the interplay between aerobic and glycolytic energy sources during exercise. This bioenergetic approach allowed us to discriminate that i) in the heavy domain, the VO_{2sc} could be the expression of a delayed adjustment of VO_2 rather than a loss of efficiency developing over time; ii) contrary to what is currently accepted, the adjustment of VO_2 in the heavy domain may be described by a slow primary component rather than by the summation of a primary plus a slow component; iii) in the severe domain the VO_{2sc} may be explained by both a prolonged metabolic shift and a true loss of efficiency over time; iv) VO_{2sc} could have different physiological underpinnings in the heavy and severe domain of exercise.

Modelling $\dot{V}O_2$ on-kinetics based on intensity-dependent motor unit recruitment and time-dependent loss of efficiency

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This study presents a new model for the VO_2 on-kinetics, with the following properties: (*i*) a progressively slower primary phase kinetics following the size-principle of motor unit recruitment, and (*ii*) a severe-domain slow component modelled as a time-dependent decrease in efficiency. VO_2 measurements from eight subjects performing step increases in work rate on a bicycle ergometer, for exercise intensities in the moderate, heavy and severe exercise intensity domains, were fitted to both the new model and the conventional 3-phase model. Model performance was evaluated with a residual analysis and by comparing Bayesian information criterion (BIC). The residual analysis showed no systematic deviations, except perhaps for the initial part of the primary phase. BIC favored the new model in the heavy domain ($p<10^{-3}$) and was unable to distinguish the models in the severe domain ($p=0.56$). Compared to the conventional 3-phase model, the proposed model distinguishes between the kinetic adaptations in the heavy and severe exercise intensity domains by predicting a delayed steady state VO_2 in the heavy domain and no steady state VO_2 in the severe domain. This prediction has important practical applications by determining when stable oxygen costs of exercise are attainable. The model might also represent a first step in defining time-dependent oxygen costs when stable energy conversion efficiency is not attainable.

Comparison between slow components of heart rate and $\dot{V}O_2$ kinetics: functional significance and practical implications for exercise prescription

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Aerobic exercise prescription is often based on the assumption of a linear relationship between pulmonary oxygen consumption ($\dot{V}O_2$) and heart rate (HR). However, we have recently demonstrated that during constant work rate exercises at different intensities, the slow component of HR kinetics occurs at lower work rate and is more pronounced than the slow component of $\dot{V}O_2$ kinetics. As a consequence, during exercise carried out at a HR slightly above that corresponding to the gas exchange threshold, both work rate and $\dot{V}O_2$ have to decrease in order to maintain HR constant. This phenomenon has been confirmed in healthy, obese patients and also in simulated microgravity condition (10 days of bed rest). Interestingly, in obese patients the decreases in work rate and $\dot{V}O_2$ were similar to those observed in young healthy physically active subjects, and were mitigated after 3-week of a multidisciplinary programme aimed at reducing body mass. The work rate decrease at a fixed HR can be considered a systemic biomarker of exercise intolerance, and may significantly affect exercise evaluation and exercise prescription, with the impossibility to identify, on the basis of HR, a specific $\dot{V}O_2$ or exercise intensity domain during a training session.
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Heterologous production and functional reconstitution of a human aquaglyceroporin of broad selectivity (AQP9)

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Establishment of a standardized, cheap and easily handled technology for high yield recombinant production and purification of aquaporins (AQPs) is essential for addressing unresolved issues including definition of the molecular pathway through which water and small neutral solutes move across membranes and significance of the AQP-mediated transport. Here, we established a reliable method to produce, reconstitute and analyze the human AQP9, an aquaglyceroporin of broad selectivity with major roles in energy homeostasis and immune system. AQP9 was efficiently produced as histidine-tagged protein (10His-AQP9) in the methylotrophic yeast *P. pastoris* and solubilized from urea- and alkali-stripped yeast membranes by the non-ionic detergent *n*-decyl-ß-*D*-maltopiranoside. 10His-AQP9 was then purified by Ni-NTA affinity chromatography, incorporated into liposomes and functionally assessed by stopped-flow light scattering. Correct protein folding was indicated by the significantly higher glycerol and water permeability shown by the 10His-AQP9 proteoliposomes compared to empty control liposomes. Correct reconstitution was also proved by the significant reduction of the glycerol and water permeability to which the proteoliposomes underwent after exposure to HTS13286, a selective inhibitor of AQP9. Production of functional AQP9 may serve as suitable and powerful tool for the discovery of clinically sustainable AQP9 blockers by structure-based and/or high-throughput screening strategies.

hiPS-cardiomyocytes for studying a specific mutation in *PITX2* gene and its role atrial fibrillation insurgence

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It is increasingly evident that genetics plays a fundamental role in the onset of atrial fibrillation (AF); GWAS studies associated *PITX2* to AF, a gene often found dysregulated in AF patients. Also, PITX2 loss of function in zebrafish has been linked to perturbations of metabolic pathways forerunning AF-like phenotypes.
We studied hiPS-derived cardiomyocytes with the M208V *PITX2* heterozygous mutation found in a young AF patient. Action potentials (AP) recorded from small clusters of pacemaker cardiomyocytes (pCMs) reveals that PITX2-pCMs are bradycardic, with a shorter AP duration and an increased AP amplitude compared to control pCMs.
To better clarify the effect in the atria, we specifically differentiated atrial-like CMs (aCMs) from PITX2 and its isogenic control (REV) lines. Since a previous work identified a gain of function of ICaL and If in hiPS-CM as possible triggering event in a genetic form of AF, we analysed these currents. Preliminary patch-clamp analysis shows no differences in ICaL and If density even if activation curve of If is negatively shifted in PITX2-aCMs. Of note, metabolism analysis with the Seahorse system revealed that PITX2-aCMs generate more ATP than REV-aCMs due to increased oxidative phosphorylation in mitochondria.
In conclusion, our preliminary data show that PITX2-aCMs have an enhanced oxidative phosphorylation and a slower beating rate, thus altering not only the electrical activity but also the metabolism of CMs.

Copper dyshomeostasis in synucleinopathies: a potential role for PLK2 kinase as a counter for a-synuclein aggregation and oxidative stress

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Parkinson's Disease (PD), see the accumulation of α-synuclein and alterations in its phosphorylation pathways, which give rise to the formation of aggregates defined Lewy's bodies, hallmarks of the pathology. In this context, the PLK2 kinase-mediated Ser-129 phosphorylation, is mainly involved in the protein clearance. Attention in recent years has been equally divided among the study of conditions promoting aggregation and the elucidation of alterations of proteolytic pathways. Conditions of impaired unfolded protein response, and alterations in proteasomal and autophagic processes are widely associated to PD onset. Copper accumulation in the central nervous system can act in a manifold manner, seeding of proteins aggregation, triggering redox cycles that generates reactive oxygen species and inhibiting degradatory pathways. SHSY5Y neuroblastoma cells were differentiated to obtain an *in vitro* model capable of assuming a dopaminergic neuron-like phenotype. Treatments with increasing concentrations of copper in the culture medium were used to replicate what observed in the pathology. In this context, PLK2 protein shows to be modulated as a consequence of our treatments, increasing α-synuclein phosphorylation, and countering the oxidative stress in neuronal cells. A better understanding of the copper-dependent alterations may therefore represent a potential route for the development of innovative clinical strategies against α-synuclein aggregation.

Endothelial integrity recovery: a honey, H_2O_2 and calcium story

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The integrity of the endothelial monolayer may be compromised by either disturbed blood flow or pathological conditions. The injured endothelium is an early event resulting in the onset of severe vascular disorders. An attractive natural product that could be used to achieve an efficient rebuilding of the endothelium is honey, a beehive product already known and used in days gone by. Honeys, in particular dark honeys, are characterized by an intrinsic production of hydrogen peroxide (H_2O_2) that confers the capability to the honey to begin the wound healing process.
The aim of this study was to determine the ability of buckwheat honey (genus *Fagopyrum*), a polyphenol-rich dark honey, to stimulate wound closure in endothelial cells. In order to evaluate the positive effects of buckwheat honey on endothelial responses, we utilized an immortalized endothelial cell line evaluating cellular responses upon honey exposure. The results highlight the positive effects of buckwheat honey on endothelial cells' biology, summarized as follow:
• buckwheat honey treatment results in H_2O_2 production outside the cells,
• H_2O_2 enters the cells through AQP3,
• cytoplasmic H_2O_2 starts two different ways to increase the $[Ca^{2+}]_i$:
  -TRPM2 channel activation entry from the extracellular space,
  -PLC-IP_3 pathway activation leading to a calcium release from the ER,
• the $[Ca^{2+}]_i$ increase contributes to PLC activation,
• PLC activation begins the MAPK pathway and the wound closure rate induction.

The neuroactive steroid ALLO mediates the dualistic role of PKCε in Schwann cell and peripheral sensory neurons

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The ε isoform of protein kinase C superfamily (PKCε) has been strongly correlated with peripheral pain modulation and neuropathic pain onset. It is renown that PKCε increases neuronal excitability, via the phosphorylation of ion channels, promoting the hyperalgesic priming of peripheral fibers. Conversely its putative physiological role in Schwann cells (SCs) is still unclear. By mean of biomolecular studies on rat primary SCs and dorsal root ganglia (DRG) neuronal cultures, we characterized PKCε activity and modulation in both cell types. We report here that the neuroactive steroid allopregnanolone (ALLO), which is synthesized in the peripheral nervous system (PNS) by SCs, modulates PKCε in DRG neurons and SCs, activating distinct mechanisms. Notably, we showed that ALLO directly downregulated PKCε in SCs. PKCε decreases cell proliferation, induces cell migration and regulates gene expression, likely through a progesterone receptor (PR)-mediated mechanism. Concurrently, ALLO upregulated and activate PKCε in DRG neurons, through paracrine mechanisms involving BDNF release and trkB activation. Overall, we report a novel mechanism of SCs – DRG neuron cross-talk in the PNS, highlighting a key role of ALLO in the modulation of PKCε and nociceptor sensitization. Our findings reveal novel physiological mechanisms and promising therapeutic targets of neuropathic pain.

Beneficial effect of hydroxytyrosol against palmitate-induced lipotoxicity in HepG2 cells: a possible role for mitochondrial fusion and fission balance

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Extra virgin olive oil (EVOO) represents the main source of vegetable fats in Mediterranean diet. It contains bioactive molecules, such as phenols to which belong hydroxytyrosol (HT), with beneficial effects on health.
Present work aimed to evaluate the effects of HT (50 and 100µM) or palmitate (PA) (100, 250, 500µM), as well as the effects of HT and PA cotreatment on cell viability, mitochondrial dynamics, intracellular lipid depots and apoptosis in hepatic (HepG2) cell culture.
Cell viability was monitored by using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay. The contents of the main proteins involved in mitochondrial fusion and fragmentation processes, as well as apoptotic markers, were detected by western blot. Finally, oil red analyses were used to study lipid accumulation in cells.
HT exposure was associated with an increase in the mitochondrial fusion marker (mitofusin 2), whereas PA exposure induced an increase in the fragmentation marker (dynamin-1-like protein) mainly in cells treated with the lowest PA dose. Noteworthy, HT/PA coincubation restored the imbalance between fusion and fission markers altered by PA. As regard lipid content and apoptotic index, PA/HT coincubation partially reverted PA exposure effects.
This study demonstrated beneficial HT effects on cell death prevention and against hepatic steatosis onset, suggesting mitochondrial involvement in both adaptive response to saturated fat induced cellular stress and HT effects.

High throughput screening using QPatch II and Qube 384 systems: electrophysiological evaluation of primary neurons, glial cells and hiPSC-derived motor neurons

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The QPatch II and Qube 384 are two automated patch-clamp (APC) systems useful for evaluating the electrophysiological properties of different cell models, with the possibility to perform high throughput compound screening for drug discovery. These systems provide high fidelity recordings which in combination with accurate, low volume applications, make them ideal for studying both voltage- and ligand- gated ion channels. With a rapidly increasing number of ion channels identified as potential therapeutic targets in neurological diseases, there is an urgency of finding valid neuronal cell models. Among these, primary brain cells and human induced pluripotent stem cells (hiPSCs)-derived neurons are promising physiological models for evaluating ion channels properties and potential drugs.
Here we used our APC systems for studying both voltage-gated (Na_V, K_V) and ligand-gated ($GABA_A$) ion channels by comparing two different brain models: *i)* primary neurons and astrocytes dissociated from mouse and rat brains, respectively; and *ii)* hiPSC-derived motor neurons.
Patch-clamp recordings require healthy membranes for high resistance sealing; however, different isolation methods may alter the membrane composition and change intracellular characteristics. Here we demonstrate that the electrophysiological properties of both brain cell models are maintained during APC recordings, confirming that both QPatch II and Qube 384 are powerful platforms for investigating brain cell models.

Axonal $GABA_A$ receptor stabilises excitability in unmyelinated nociceptor axons secondary to NKCC1 shift in E_{Cl^-}

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Unraveling the mechanisms of nociception under physiological conditions may provide novel findings for the comprehension of the physiopathology of neuropathic pain onset and chronicisation.
In this light, we focused our efforts on the study on GABAergic system modulation of excitability of peripheral C-fibers axons, by means of electrophysiological *ex vivo* compound action potential recording on wildtype and transgenic mice nerves, in combination with molecular biology techniques. We report here that $GABA_A$ receptor ($GABA_AR$) mediates depolarizing currents in the unmyelinated axons of somatosensory C-fibre and that the magnitude and time course of $GABA_AR$ responses are coupled to NKCC1 activity. To examine the physiological role of axonal $GABA_AR$, C-fibres were subjected to a sustained frequency challenge. We found that the amplitude of axonal GABA responses was increased secondary to an NKCC1 mediated shift in E_{Cl^-}. That $GABA_AR$ activation rose the axonal conduction velocity of C-fibres and lastly, we demonstrated that axonal $GABA_AR$ was activated by endogenous ligands. Here we posit that axonal $GABA_AR$ counteracts the activity-dependent loss of excitability in C-fibre nociceptors after NKCC1 increase of axonal Cl^- gradient, allowing C-fibres to sustain firing. Regulation of axonal Cl^- concentration and $GABA_AR$ may be an effective means to regulate C-fibre hyperexcitability, that was found in some patients with chronic peripherally-mediated pain condition.

Impact of AQP4 supramolecular organization in migrating reactive astrocytes

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Brain astrocytes express the water channel aquaporin-4 (AQP4) as molecular aggregates, known as Orthogonal Arrays of Particles (OAPs). The aim of this study is to investigate the role of OAPs in astrocyte pathophysiological responses tuned upon pro-inflammatory conditions, with a focus on migration. To this end, we promoted reactive gliosis *in vitro* by treating WT and OAP-null primary-cultured mouse astrocytes for 7 days with 10 ng/ml TNF-α and IL-1β. Astrocyte activation was assessed by RT-qPCR of pro-inflammatory markers (IL-1β, TNF-α, CCL5, CXCL10) whose expression was found to be 3-fold higher under activating conditions. We evaluated changes in the cytoskeletal arrangements of fixed cells stained for F-actin after 7 days of stimulation by morphometric analysis that revealed that astrocytes undergo a 2-fold increase in the outline/area ratio due to the transition from polygonal to hypertrophic morphology. However, no differences in the shape change were detected between the two genotypes. Wound healing assays performed in reactive astrocytes and wt-AQP4 and OAP-null-HeLa cells show that the speed of migration is greatly impaired in OAP-null inflamed astrocytes (WT=8,643±0,07 um/hr;OAP-null=2,171±0,06 um/hr), as in HeLa lacking AQP4-aggregates (wt-AQP4= 21.05 um/hr±0.04;OAP-null=15.06 um/hr±0.05). Altogether these findings prove that, besides water homeostasis, AQP4 supramolecular organization is involved in the mechanobiology of astrocytes in neuroinflammation responses.

Kidins220/ARMS mediates astrocyte developmental switch in BDNF sensitivity, calcium signaling and neuron-astrocyte communication

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Astroglial cells are key to maintain nervous system homeostasis and regulate neuronal circuit formation and activity. Neurotrophins are growth factors with pleiotropic effects on neuronal and astrocyte physiology. Kidins220/ARMS (Kinase-D interacting substrate of 220 kDa/Ankyrin repeat-rich membrane spanning) is an effector of neurotrophin signaling in neurons, however, its role in glial cells remains largely unknown.
In this work, we compared the signaling competence of embryonic and postnatal primary cortical astrocytes exposed to brain-derived neurotrophic factor (BDNF) and found a shift from a kinase-based response in embryonic cells to a Ca^{2+}-based response in postnatal cultures. In the absence of Kidins220, astrocytes are characterized by reduced full-length and truncated TrkB expression and impaired BDNF-dependent kinase and Ca^{2+} pathways. Kidins220 ablation induces defects in Ca^{2+} signaling linked to altered store-operated Ca^{2+} entry and overexpression of the transient receptor potential channel TRPV4. Moreover, embryonic Kidins220$^{-/-}$ astrocytes are more sensitive to genotoxic stress and display altered metabolic balance. Last, Kidins220 expression in astrocytes is required for the maturation of co-cultured neurons.
Altogether, our data contribute to the understanding of the complex role played by astrocytes within the central nervous system and identify Kidins220 as a novel actor in the increasing number of pathologies characterized by astrocytic dysfunctions.

The pathophysiology of retina neurons through the eye of the fruit fly *Drosophila melanogaster*

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Drosophila melanogaster (*Dm*) is a powerful experimental *in vivo* tool to investigate complex diseases, including visual degenerations. Dm is characterized by high gene conservation and mechanisms, and structural/functional similarities with vertebrate visual system. We have recently used two Dm mutant strains to explore eye homeostasis in the absence of functional full-length dystrophin. Similarly to mdx mouse, the classic model of Duchenne muscular dystrophy, retinas of Dm mutants displayed altered neuronal architecture, apoptosis, defective autophagy and altered light response. Interestingly, boosting of autophagy by rapamycin prevented neurodegeneration and visual ability indicating that autophagy is a crucial mechanism for preserving vision, and that dystrophin is required for synapse stabilization and neuronal survival. Using a different approach, we observed that adult wild-type Dm fed with high-sucrose regimens had typical features of the initial stage of diabetes. Hyperglycemic flies showed a decrement in the responsiveness to light and neurodegeneration of photosensitive components as well as neuronal apoptosis, oxidative stress and dysfunctional autophagy, as earlier described in vertebrate models. Noteworthy, antioxidant nutraceuticals treatment positively affected the visual system of hyperglycemic flies at structural/functional levels. Taken together, these evidences support Dm as a proper in vivo model for the study of retina functions and dysfunctions.

MicroRNA-155 is a target to slowing down retinal neuron degeneration in a Retinitis Pigmentosa mice model

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Retinitis Pigmentosa (RP) is an orphan disease characterized by progressive degeneration of photoreceptors and abnormalities in retinal pigment epithelium. Several evidences identified microRNAs (miRNAs) e.g. miR-155, as deregulated in development and progression of RP. In this context, targeting miR-155 could represent an innovative and attractive therapeutic strategy. The aim of this study is to evaluate the pharmacological activity of an innovative eye drops formulation containing antimiR-155 (molecules targeted miR-155) loaded on nanosized colloidal carrier, nanosponges (NS).
In order to evaluate the delivery and functional activity of antimiR-155/NS formulation in an animal model of autosomal recessive RP (rd10), we administered antimiR-155/NS from postnatal day (P) 18 up to P30 in treated eye and antimiR-scramble/NS in control eye of the same animal. The *in vivo* delivery was evaluated by using NS coniugated with fluorophore and fluorescence microscopy evaluation of retinal tissues. Moreover, real-time PCR was used to evaluate miR-155 levels after treatment.
Our results show that the treated eye has an improvement of retinal function (electroretinogram recording), indicating a reduction in degenerative processes attributable to a reduction of inflammatory pathway (real-time PCR and western blotting) in retinal tissue. Altogether, these data suggest that NS-Anti-miR-155-5p could represent a potential therapeutic agent for RP.

Mapping human brain network(s) using SEEG: challenges and breakthroughs

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Correlations between neuronal signals are ubiquitous characteristics of large-scale brain dynamics. Phase correlations, in particular, facilitate neuronal communication when they are present with optimal lag that aligns high excitability periods of targets with the conduction-delayed spikes from a sources. In the last decades, Stereo EEG is being increasingly adopted in advanced research settings aiming at mapping interaction between brain regions. In this talk, I will revise state-of-the-art approaches for pre-processing SEEG data including optimal referencing approaches and accurate channel localization in respect to individual anatomy. Then I will discuss the application of SEEG for the characterization of large-scale phase synchronization networks. I will present recent evidence of long-range phase synchronization of high-frequency oscillations in the human brain as well as genuine zero-phase lag coupling of brain oscillations. Our results indicate that that even in the supposedly highly variable resting state, emergent brain dynamics are characterized by an organized mosaic of inter-areal phase relationships.

Tonic somatosensory responses as the neural fingerprint of tactile awareness

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The quest about the neural mechanisms sustaining sensory awareness has met several hurdles on its way, including the poor spatial and temporal resolution offered by non-invasive recording techniques, and the consequent inconsistencies in localization results and mechanistic descriptions.
To date, both theoretical accounts and empirical studies pointed to recurrent processing as a condition necessary or even sufficient for instantiating and sustaining perceptual awareness. Taking advantage of invasive intracerebral recordings performed on surgical or pre-surgical patients, recent studies refined not only the localization of neural substrates possibly underlying the instantiation of sensory awareness, but also the temporal dynamics of their activation.
This talk will provide an overview of the latest research conducted via stereo-electroencephalography –sEEG– on the neural correlates associated to somatosensory awareness. In particular, we will first focus on the identification of cortical activities whose temporal dynamics closely matches that expected for recurrent activity, i.e. non-earliest, long-lasting and reliable responses, following tactile stimulus delivery. Subsequently this talk will show how the spatial localization of such activity (mostly perisylvian) – referred as the *tonic activity* - co-localizes with the lesional mapping of tactile disorders (e.g. tactile extinction), settling this spatio-temporal feature as the ideal neural correlate of tactile awareness.

Loss of differentiation and complexity in the sleeping human brain: a multi-scale analysis

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Previous studies suggest that local sleep-like neuronal bistability, can be responsible for the collapse/emergence of global patterns of complex interactions among brain areas. Here, we link these two scales by combining intracerebral single pulse electrical stimulation (SPES) with simultaneous invasive recordings and scalp high-density EEG.
This work includes data collected during presurgical evaluation from 16 epileptic subjects. Recordings of simultaneous SEEG and hd-EEG activity were combined with SPES (5mA, 1/0.5ms,0.5Hz) during both wakefulness and NREM sleep. 100 stimulation sessions were performed by delivering SPES at rest in different areas. We show that:
1) The amplitude of the scalp-EEG response to SPES well correlates with the underpinning intracerebral activity ($r2 = 0.72$, $p<0.05$).
2) The overall complexity, as assessed by the Perturbational Complexity Index was significantly higher in wakefulness with respect to NREM ($p<0.05$).
3) The differentiation of the response to SPES, evaluated by performing the Principal Component Analysis across all sessions and comparing the ensuing number of components across states, was significantly higher in wakefulness with respect to NREM over the parietal areas ($p<0.05$).
4) The reduction of complexity and differentiation was marked by the occurrence of an evoked slow wave (0.5-4Hz) associated with a suppression of high frequency power (>20Hz) that was significantly more prominent in NREM with respect to wakefulness ($p<0.05$).

Direct electrical stimulation of the premotor cortex shuts down awareness of voluntary actions

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A challenge for neuroscience is to understand the conscious and unconscious processes underlying construction of willed actions. We investigated the neural substrate of human motor awareness during awake brain surgery. In a first experiment, awake patients performed a voluntary hand motor task and verbally monitored their real-time performance, while different brain areas were transiently impaired by direct electrical stimulation (DES). In a second experiment, awake patients retrospectively reported their motor performance after DES. Based on anatomo-clinical evidence from motor awareness disorders following brain damage, the premotor cortex (PMC) was selected as a target area and the primary somatosensory cortex (S1) as a control area. In both experiments, DES on both PMC and S1 interrupted movement execution, but only DES on PMC dramatically altered the patients' motor awareness, making them unconscious of the motor arrest. These findings endorse PMC as a crucial hub in the anatomo-functional network of human motor awareness.

Therapeutic potential of dietary nitrate in pregnancy

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Nitric oxide (NO) plays a vital role in mediating maternal vascular adaptation to pregnancy, as well as being important for fetal and placental development and function. NO is derived either via the classical pathway involving nitric oxide synthase (NOS) enzymes, or the 'alternative pathway', involving sequential reduction of dietary nitrate acquired from foods such as green leafy vegetables and beetroot.
In non-pregnant individuals, supplementation with dietary nitrate in the form of beetroot juice has been shown to improve cardiovascular function, reducing blood pressure (BP), enhancing endothelial function and improving blood flow. In our studies, we aim to determine the therapeutic potential of dietary nitrate supplementation in pregnancy. To address this, we use a range of preclinical models including primary human tissues and pregnant mice, and have also started to translate this approach into small clinical trials in pregnant women.

Beneficial effects of grape seed extract polyphenols in pregnancy

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The maternal vascular endothelium is an important target of factors triggered in pregnancies complicated by hypertension. Increased oxidative stress during pregnancy, may worsen the underlying maternal disease and predispose to the development of subsequent maternal and fetal cardiovascular disorders. Therapeutic interventions aimed at reducing oxidative stress continue to be an expanding area of research in a variety of diseases, including gestational pathologies.
Polyphenols extracted from grape seeds (GSEP) provide a natural source of antioxidants. Preclinical and clinical studies demonstrated protecting effects of GSEP against endothelial dysfunction and hypertension. This work will present data from our animal model of maternal hypertension associated with vascular dysfunction, used to investigate biological effects of GSEP on maternal key target blood vessels and pregnancy outcomes.

Effects of bisphenol A on maternal uterine vasculature and on feto-placental development in rat

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Bisphenol A (BPA) is an endocrine disrupting chemicals (EDC) present in the human body mainly, coming from the environment, mainly introduced through the diet, since BPA is a component of both polycarbonate and of epoxy resin used in food containers respectively plastic and cans. BPA interferes with the endocrine system and impairs human health. Its high concentrations observed in the blood of pregnant women, in amniotic fluid, placenta and umbilical cord are associated with complication of women's reproductive health.
We showed for the first time that BPA compromised the rat placenta-fetal unit development by acting on the uterine vasculature, impaired endothelial function and compromised the uterine vascular remodelling associated to pregnancy.

Metabolic heart adaptation in fetal rats exposed to Bisphenol A

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Dietary exposure to Bisphenol A (BPA), an endocrine disruptor chemical, in critical periods, such as uterine life, can affect the proper development and function of multiple organs, as well as the control of the energy balance and homeostasis of nutrients in the adult offspring. We observed in a murine model that maternal dietary exposure to this endocrine disruptor alters the placental glucose transporter 1, and therefore the level of the sugar in the fetal compartment.
The fetal heart consumes more energy than any other organ and it has a peculiar metabolic profile in terms of energy substrate utilization during its development, with glucose as the key substrate for its metabolism. However, during the transition from fetal to postnatal life, the heart shifts from glycolysis to fatty acid beta-oxidation.
We used female rats fed with a diet containing BPA to investigate the effect of the chemical on fetal heart metabolism. In our study, we detected a dysregulated expression of proteins involved in the glucose and fatty acids transport and metabolism in BPA exposed pups. Similar effects were observed in acute exposure to the chemical using an *in vitro* model of fetal heart explants cultures. On the other hand, we did not observe any alteration in the major pathway related to cell fate suggesting a metabolic adaptation without any manifest damage of the fetal heart. The prenatal exposure to BPA, altering heart metabolism, could compromise cardiovascular performance in adulthood.

β_3-adrenoreceptor participates in the sympathetic regulation of the renal acid-base homeostasis

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Sympathetic nervous system regulates several renal functions. We previously demonstrated that the β_3-adrenoreceptor (β_3-AR) is expressed in mouse collecting duct principal cells and its stimulation promotes antidiuresis. Here, we report the β_3-AR is also expressed in the collecting duct intercalated cells (ICs) regulating the acid-base homeostasis in the kidney. Co-localization of β_3-AR either with H^+-ATPase or Cl^-/HCO_3^- exchanger pendrin showed β_3-AR expression in type A, type B, and in non-A non-B ICs in mouse kidney. Urine pH of β_3-AR knock-out (ko) mice was significantly higher compared with wild type (wt) mice (β_3-AR ko 5,89 ± 0,011 vs wt 5,704 ± 034). Of note, the abundance of H^+-ATPase was significantly decreased in the kidneys of these mice, supporting the idea that H^+ secretion is partially blunted.
In cultured renal cells expressing β_3-AR and challenged by intracellular acid load, selective β_3-AR agonism induced a 2.5 fold increase in H^+-ATPase activity when compared to resting cells. This effect was effectively prevented upon treatment with either a selective β_3-AR antagonist or the H^+-ATPase inhibitor bafilomycin.
Moreover, the increase in H^+-ATPase activity elicited by β_3-AR agonism was abolished in the presence of the PKA inhibitor H89, demonstrating the involvement of the cAMP/PKA pathway. Taken together these results suggest a novel physiological role of β_3-AR in the sympathetic control of renal acid-base homeostasis.

Optical stimulation of endothelial colony forming cells plated on light-sensitive conjugated polymers induces a TRPV1-mediated increase in intracellular Ca^{2+} concentration

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Endothelial colony forming cells (ECFCs) represent the only endothelial precursor showing *in vitro* proliferation and *in vivo* vessel formation. ECFCs are promising candidates for autologous cell-based therapy of ischemic disorders, due to their regenerative capacity and clonogenic potential. Manipulating intracellular Ca^{2+}-dependent pathways that finely tune angiogenic activity could further boost ECFC's regenerative potential. Optical excitation of the light-sensitive conjugated polymer, regioregular Poly (3-hexyl-thiophene) (rr-P3HT), was recently shown to stimulate ECFC proliferation and tube formation upon the activation of the non-selective cation channel Transient Receptor Potential Vanilloid 1 (TRPV1). Herein, we adopted a multidisciplinary approach, ranging from Ca^{2+} imaging to genetic manipulation, to analyse TRPV1-dependent increase in intracellular Ca^{2+} concentration induced by light in ECFCs plated on rr-P3HT thin films. These data clearly show that optical stimulation of rr-P3HT results in TRPV1-mediated intracellular Ca^{2+} signals in ECFCs. The Ca^{2+} response to light is triggered by hydrogen peroxide, which can be produced at the interface between rr-P3HT thin films and the cell membrane and then directly gate TRPV1. These data provide the evidence that TRPV1 may serve as a decoder that translates optical excitation into pro-angiogenic Ca^{2+} signals in ECFCs plated on rr-P3HT.

Placenta-brain axis: focus on astrocytes

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Recent studies show that the placenta plays an active role in the physiology of the central nervous system (CNS). This organ directs fetal neurological development and structural/functional changes in the maternal brain during pregnancy are also attributable to the placenta. The placenta releases a wide range of molecules, both into the maternal and fetal circulation, and many of these can easily reach the CNS.
We hypothesized that the placental secretome regulates the physiology of a particular type of glial cell: astrocytes. These cells are important elements for the function and survival of neurons, but also capable of promoting inflammation and neurodegeneration.
We performed *in vitro* cultures of human placenta explants to assess whether placental insults produce alterations in astrocyte physiology or whether the neurotoxic function of activated astrocytes towards an inflammatory phenotype are mitigated by exposure to the placental secretome. Activated astrocytes up regulated the expression of pro-inflammatory chemokines, which returned to physiological levels when the astrocytes were treated with conditioned media from placental explants. Exploiting the physiological processes of pregnancy and using the placenta as a working model, we found that molecules present in placental secretome can mitigate the inflammatory behaviour of astrocytes giving the possibility to develop drugs useful to control/ameliorate acute neurological injuries and/or chronic CNS diseases.

Considerations around the SARS-CoV-2 Spike protein, airborne particulate matter and SARS-CoV-2 brain infection

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Growing evidence indicates that different types of coronaviruses not only affect the respiratory system, but they might also invade the central nervous system (CNS). However, very little evidence has been so far reported on the presence of COVID-19 in the brain. We assessed the SARS-CoV and SARS-CoV-2 Spike protein (S protein) sequence, structure, and electrostatic potential using computational approaches. We found that the SARS-CoV-2 S protein is slightly more positively charged than that of SARS-CoV since it contains four more positively charged residues and five less negatively charged residues which may lead to an increased affinity to bind to negatively charged regions of other molecules through nonspecific and specific interactions. Assuming that a fraction of aerosols remains infective, “droplet-nuclei” might contribute to airborne transmission of the virus, particularly in poorly ventilated and crowded indoor spaces. In contrast to the inhalation mode of viral transmission through airborne respirable droplets, here we speculate an additional role for settled and airborne particulate matter (PM) not only in viral transmission through inhalation and ingestion, but also in promoting immunity through antigen delivery, adjuvanticity and trained immunity. These results might be useful for understanding the mechanism of cell entry, blood-brain barrier crossing, and clinical features related to the CNS infection by SARS-CoV-2.

Solid supported membrane-based electrophysiology (SSME) meets SGLT1 and GAT1

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Transporter assays are often limited by the availability of labeled substrates and lack real-time data. Here, we developed functional assays to characterize the human Na^+/Cl^-/γ-aminobutyric acid (GABA) and Na^+/glucose co-transporters - GAT1 and SGLT1 - using solid supported membrane-based electrophysiology (SSME).
Transport and PSS electrogenicity in SGLT1 and GAT1 triggered by voltage steps is postulated to be a result of transitions within the sugar and GABA-free carriers, e.g. the alternating access of the charged sodium binding sites within the empty carrier. SSME utilizes membrane vesicles at 0 mV and the transport cycle is triggered by applying a substrate concentration gradient as the main driving force. Using SSME, we observed substrate-induced PSS currents, most likely representing conformational transitions within the substrate-loaded carrier, which are not observed with conventional electrophysiology. We examined the impact of different driving forces on influx, efflux, and PSS currents, focusing on sodium gradients and membrane voltage. We found that internal accumulation of sodium strongly reduces Vmax, rendering sodium release rate limiting at 0 mV. Application of membrane voltage only affected the apparent KM in SGLT1, but Vmax in GAT1. We also found that transport properties in GAT1 mediated influx and efflux modes are highly asymmetric, while SGLT1 has similar properties for influx and efflux.

Key words: SSME, transporters, GAT1, SGLT1

Climate change and heat illness risk: a matter for physiologists

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Climate change effects include an increased risk due to heat events during the hot months that have been shown to be characterized by more intense and long-lasting hot ambient temperatures. Starting with the 2003 heatwave that hit Europe and resulted in 15000 excess deaths in France, attention has been dedicated to understand the risk factors and to suggest countermeasures. Temperature regulation represents one of the key mechanisms to maintain body homeostasis and it is the result of a complex integration between the nervous system and other systems, including the afferent sensory pathways and the efferent pathways inducing cardiovascular, sweating and behavioral adaptations. Physiological responses to heat have been investigated in both healthy and vulnerable individuals, ranging from the field of sports science to more clinical studies. Among the vulnerable individuals, people with neurological diseases are often characterized by impaired thermoregulation due to dysautonomic symptoms, and might be at a higher risk of heat-illness. Despite some of these impairments have been described, more studies are needed to convey basic laboratory findings to ecologically valid conditions following a translational approach in order to better identify signals of increased risk of heat illness and develop new preventive and mitigation strategies by applying physiological models (e.g., the predictive heat strain model) in real life conditions.

Effect of short-term fructose-rich diet on brain: what is reversible and what persists after sugar removal from the diet?

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Young age is characterized by high consumption of processed foods and drinks rich in fructose that can promote overweight, but also brain dysfunction. The aim was to (a) clarify brain effects resulting from short-term fructose consumption in juvenile age; (b) verify whether fructose-induced alterations can be rescued or persist after sugar removal from the diet. Young rats were fed a fructose-rich or control diet for 3 weeks. Half of fructose-fed rats were then fed a control diet for a further 3 weeks to study the possible persistence of brain changes. Mitochondrial bioenergetics, glucose transporter-5, fructose and uric acid levels, oxidative status, inflammation, as well as neurotrophins, survival factors and markers of synaptic function were evaluated in the hippocampus and frontal cortex. The fructose-rich diet induced mitochondrial dysfunction and oxidative stress, associated with neuroinflammation, decreased Neurofilament-M and post-synaptic density protein 95. These alterations, except for increases in nitrotyrosine, were recovered by returning to a control diet. Unlike the hippocampus, alterations in brain-derived neurotrophic factors, Akt and Erk phosphorylation extent, and acetylcholinesterase activity persisted in the frontal cortex, even after the switch to control diet. Given the increasing consumption of fructose-rich foods in young populations, these results highlight the risk arising from brain persistent alterations even after the end of a sugar-rich diet.

Caloric restriction mitigates kidney fibrosis and apoptosis in an aged and obese rat model

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Caloric restriction represents a powerful intervention for extending health span and lifespan in several animal models, from yeast to primates, including human. Caloric restriction has been found to induce cardiometabolic adaptations associated with improved health and to delay the onset and progression of kidney disease in different species, particularly in rodent models. In both aging and obesity, fibrosis is a hallmark of kidney disease, and epithelial–mesenchymal transition is a key process that contributes to fibrosis and the decline of renal function during aging.
In this study, utilizing kidney tissues of aged and obese rat model, we evaluated the effect of long-term (6 months) caloric restriction (-40%) on mesenchymal (N-cadherin, Vimentin, Desmin and α-SMA), antioxidant (SOD1, SOD2, Catalase, GSP1 and Hsp70), inflammatory (YM1 and iNOS) markers and apoptotic/cell cycle (BAX, BCL2, pJNK, Caspase 3 and p27) pathways by western blot analysis. By histological techniques, we also evaluated renal interstitial fibrosis.
Our results clearly showed that caloric restriction promotes cell cycle division, reduces apoptotic injury and fibrosis phenotype through the inflammation attenuation and leukocytes infiltration.

Effects of Very Low Calorie Ketogenic Diet on autonomic nervous system activity

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Caloric restriction is a valid strategy to reduce visceral adipose tissue in obesity. Salivary amylase is an enzyme cleaves large starch molecules into dextrin and subsequently into smaller maltooligosaccharides and its production is modulated also by central nervous system. The study of this variation is considered an indirect measure of cardiac autonomic function, the so-called heart rate variability (HRV), and represents an useful tool for evaluating sympathetic and parasympathetic modulation of the heart. The current study aimed to evaluate the effect of a very low-calorie ketogenic diet (VLCKD) in a population of obese patients on autonomic nervous system. We evaluated in obese population before and after 8 weeks of VLCKD intervention, anthropometric and biochemical parameters, salivary amylase by ELISA-test and HRV analysis. Salivary amylase levels and HRV significantly increased after dietary treatment, and positively correlates to each. VLCKD exerts a positive effect on salivary amylase and HRV, ameliorating adiposity and blood biochemical parameters. In a brief term, this dietary intervention improves the autonomic nervous system activity. Finally, to the best of our knowledge, this is the first study about the effects of VLCKD upon the autonomic nervous system, supporting the usefulness of such a therapeutic intervention in promoting reduction in the individual burden of disease.

Ablation of UCP3 affects visceral white adipose tissue functionality

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Mitochondrial Uncoupling protein 3 (UCP3) plays a role in energy homeostasis, however, its function in visceral adipose tissue (vWAT) remains largely unknown. We studied the impact of UCP3 ablation on vWAT mitochondrial oxidative capacity and endoplasmic reticulum stress (ER stress), since dysfunctions on either organelle can disrupt adipocytes homeostasis and lead to metabolic derangements (insulin resistance, inflammation, lipolysis, adipokines secretion). As animal model we used wild type and UCP3 Knockout (KO) mice housed at thermoneutrality.
vWAT from KO mice showed impaired cytochrome oxidase activity (index of maximal tissue oxidative capacity), increased mitochondrial free radicals levels, and increased ER stress protein markers (GRP78/BIP and calnexin). vWAT from KO mice displays metabolic derangements such as: a blunted response to the *in vivo* insulin administration (revealed by the reduced phosphorylation of ser-473 Akt/PKB), increased lipolysis (assessed by glycerol release by the tissue) and inflammation (revealed by increased tissue MCP-1 and TNFα levels). Adipokines proteins array revealed that UCP3 ablation leads to variations in tissue and serum adipokines levels; among these, adiponectin resulted significantly reduced, thus suggesting changes in vWAT endocrine signalling.
As a whole these data suggest that in vWAT UCP3 ablation leads to mitochondria dysfunction and ER stress contributing to adipocyte dysfunction, and a thwarted metabolic homeostasis.

Long-term passive static stretching and vascular function

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Stretching effects on blood flow (Q), shear rate (Y), and vascular function in the stretched muscle feeding arteries have been already investigated. Few data are available on vascular adjustments induced by long-term PS training. We investigated the effects of PS training on vascular function and stiffness of the involved (femoral and popliteal) and uninvolved (brachial) arteries. Hypothesis was that PS-induced changes in Q and Y would improve central and local Q control mechanisms. 39 participants were randomly assigned to bilateral (n=14), monolateral (n=13) or no PS training (n=12). Vascular function was measured before and after 12 weeks of knee extensor and plantar flexor muscles' PS training by single passive limb movement and flow-mediated dilatation (FMD). Central (carotid-femoral artery, PWVCF) and peripheral (carotid-radial artery, PWVCR) arterial stiffness was measured by pulse-wave velocity (PWV), together with systolic (SBP) and diastolic (DBP) blood pressure. PS increased the femoral Q , popliteal and brachial artery FMD%, by 30%, 25% and 8%, in both PS training groups (P<0.05), and decreased PWVCF, PWVCR, SBP and DBP (−25%,−17%,−4% and−8%, respectively;P<0.05). No changes occurred in controls. These adaptations are suggestive of modifications in central and local Q control mechanisms. PS-induced improvements had a short duration as some vascular function parameters returned to baseline within 6 weeks of PS training cessation.

The role of the endothelium in the hyperemic response to passive leg movement: looking beyond nitric oxide

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Passive leg movement (PLM) evokes a nitric oxide (NO)-mediated increase in blood flow that declines with age and disease. PLM is becoming increasingly accepted as an assessment of vascular function. However, a substantial PLM-induced hyperemic response is still evoked despite NO synthase (NOS) inhibition. Therefore, in 9 young men (25±4 yrs), we aimed to determine if the combination of two endothelium-dependent vasodilators, prostaglandin (PG) and endothelium-derived hyperpolarizing factor (EDHF), account for the remaining hyperemic response when NOS is inhibited. The leg blood flow (LBF) response to PLM and sPLM following the intra-arterial infusion of N^G-monomethyl $_L$-arginine (L-NMMA), to inhibit NOS, was compared to the combined inhibition of NOS, cyclooxygenase (COX), and cytochrome P450 (CYP450) by L-NMMA, ketorolac tromethamine (KET), and fluconazole (FLUC), respectively. NOS inhibition attenuated the LBF (LBF_{AUC}) response to both PLM (control: 456±194, L-NMMA: 168±127 ml) and sPLM (control: 185±171, L-NMMA: 62±31 ml). The combined inhibition of NOS, COX, and CYP450 (i.e. L-NMMA+KET+FLUC) did not further attenuate the hyperemic responses to PLM (LBF_{AUC}: 271±97 ml) or sPLM (LBF_{AUC}: 72±45 ml). PG and EDHF do not contribute to the non-NOS-derived NO-mediated, endothelium-dependent, hyperemic response to either PLM or sPLM in young men. These findings add to the mounting evidence and understanding of the pathways assessed by the PLM and sPLM vascular function tests.

Effects of passive leg movement-based training: a vascular serendipity

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Background. Despite vascular dysfunction and associated disorders are remarkable side-effects of chronic bed-rest, potential treatments such as passive mobilization have been only theorized. The aim of the study is to investigate the effects of a passive mobilization treatment on vascular function in older people who are chronically bedridden.
Methods. 45 older people who are chronically bedridden, were randomly assigned to a Treatment group (n=23) or control group (CTRL, n=22). Treatment group performed 4 weeks of repeated passive mobilization, two times a day (30 mins, 5 t/w). The passive mobilization was performed in one leg (treated leg, T-leg Vs ctrl-leg), and consisted in the passive knee flexo/extension at 1 Hz. CTRL continued to be treated as usual. Primary outcome was the variation of peak blood flow (ΔPeak) measured at the common femoral artery during single passive-leg movement test (sPLM).
Results. Treatment group increased ΔPeak in both legs (+91 ml/min, in T-leg and +25 ml/min, in ctrl-leg), In the CTRL group no difference between pre- to post-treatment was found.
Conclusion. Older people who are chronically bedridden reported significant improvements vascular function after 4-week of passive mobilization. This treatment generated both peripheral and systemic positive adaptations and it might be included in standard clinical practice as an effective strategy to treat vascular dysfunction in subjects with severe mobility limitations.

Vascular function and blood flow regulation in patients with heart failure

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Patients with heart failure (HF) are characterized by exercise intolerance, premature fatigue, and limited oxygen delivery and utilization, perhaps as a consequence of diminished peripheral vascular function. This presentation will explore the impact of disease-related changes in peripheral vascular control in HF patients with both reduced (HFrEF) and preserved (HFpEF) ejection fraction, and highlight recent studies aimed at strategies for restoring vascular health in these patient groups.

Single neurons and circuits of the cerebellum

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Several experimental approaches were used in the last decades to characterize neuronal functions, from the molecular to connectivity levels. While single cell electrophysiology remains the gold standard to investigate cellular and subcellular mechanisms, network investigation requires technological advancements only recently achieved. In the cerebellum, neuronal properties endow the network with emerging functions, impacting on other brain regions through extensive connectivity. Cutting-edge techniques as multielectrode arrays and miniscope devices in behaving mice proved to be fundamental. The huge increase in data complexity requires computational models to interpret the results and drive further research. By using a new neuroinformatic framework (Brain Scaffold Builder), for the first time, an entire module of mouse cerebellar cortex was reconstructed using morphologically realistic multi-compartmental neuron models. Multiple connection rules (as probability clouds, touch detection, voxel intersection) were used to generate the connectome, unifying scattered experimental data into a coherent construct. Baseline and sensory-burst stimulation were used for functional validation against *in vivo* data, monitoring the impact of subcellular and cellular mechanisms on spatio-temporal signal processing. The use of realistic properties in the cerebellar network model provides a new “ground truth” on circuit organization able to predict neural dynamics and behavioral correlates.

Detailed cerebellar models in whole-brain multiscale cosimulations

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Unveiling the causal relationships of neural mechanisms across scales is fundamental to understand brain functioning and diseases. To address this issue, multiscale cosimulations are being developed. In cosimulations, mean-field models of multiple brain regions are interfaced with detailed models of specific microcircuits, to study the brain at different granularities and bridge the gap across scales [Meier et al, 2021].
Here, multiscale cosimulations are developed to investigate the role of complex cerebellar dynamics in whole-brain activity. A detailed model of a cerebellar microcomplex reconstructed and validated on morphological and electrophysiological data [De Schepper et al, in prep] is embedded in The Virtual Brain (TVB) [Ritter et al, 2013], with the long-range connectome derived from the Allen Mouse Brain Connectivity Atlas [Oh et al, 2014]. The olivocerebellar model is simulated as a spiking network with neural populations represented by ad-hoc optimized point neurons (NEST simulator). Global coupling and bi-directional interfaces between NEST and TVB signals are tuned to match data on mouse resting-state functional connectivity and cerebellar basal spiking rates. Cerebellum-driven protocols are exploited to investigate how complex propagation and spatio-temporal processing of sensorimotor signals in the cerebellar microcircuit impacts on whole-brain dynamics. The tool will be used to investigate pathologies involving specific cerebellar microcircuit alterations.

Exploring the cerebellum in humans through MRI recordings and virtual brain modelling

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The activity of several interconnected networks, comprising both cerebral and cerebellar structures, contributes to generate global brain dynamics. The relationship between brain structure, function and dynamics can be investigated using appropriate modeling approaches integrating multiscale empirical data. The Virtual Brain (TVB) is an advanced data-driven model that simulate subject-specific brain dynamics starting from their structural and functional MRI connectivity. For the first time, we introduced cerebellar nodes and interconnecting tracts to demonstrate the impact of cerebro-cerebellar loops on brain dynamics. In particular, cerebral dynamics were simulated either including or excluding cerebro-cerebellar connectivity in 10 healthy subjects from the ConnectomeDB. Our findings revealed that the matching between the empirical and simulated functional connectivity, expressed as Pearson correlation coefficient, was significantly improved when including the cerebro-cerebellar loops. Despite this result suggests a key role of the cerebellum on brain dynamics, it is a preliminary result because the best strategy to reconstruct effective structural connectivity and to define the models generating specific local activity in the nodes are still missing. Tackling these challenges is expected to further improve the predictive power of functional brain activity simulations in explaining the cerebellar role either in in physiological and pathological conditions.

Virtual brain modelling in cerebellar ataxic patients

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Pediatric cerebellar ataxias (PCAs) are neurological disorders characterized by impairment in motor coordination. Surprisingly, within PCA, patients with Joubert syndrome may show postural motor behaviour comparable to that of healthy subjects, probably due to the non-progressive evolution of the pathology. Indeed, remaining brain areas could cope with a stable lesion by neural plasticity. To uncover the neural substrates involved in functional compensation, we simulated whole-brain dynamics starting from a multimodal dataset within "The Virtual Brain (TVB)" framework, including or excluding the cerebro-cerebellar connections. One subject affected by Joubert syndrome and two with slow-progressive ataxia underwent diffusion and resting state fMRI to create specific structural and experimental functional connectomes (expFC). Brain dynamics were simulated for 6 minutes. Preliminary data highlighted that Pearson correlation coefficients (prediction power) between expFC and simulated FC strongly decreased not only when cerebro-cerebellar connectivity was excluded from the generation of cerebral activity, but also in slow-progressive patients. This result supports the hypothesis that in patients with non-progressive disorder, the cerebellum may create new neural pathways providing compensatory strategies in brain networks. Future studies are warranted to focus on specific neural candidates that could be involved in the functional compensation.

Functional characterization of a novel *HCN2* variant associated with progressive epileptic encephalopathy in neonatal rat neurons

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Hyperpolarization-activated cyclic nucleotide-gated channel (HCN) missense mutations play an important pathogenic role in different forms of epilepsy and we are still far from understanding the mechanisms responsible for the variety of phenotypic aspects of the patients, in particular epilepsy and cognitive defects. Here we present the case of a patient with a congenital progressive epileptic encephalopathy characterized by severe developmental delay, ataxia, dystonia and cerebral visual impairment, harbouring a mutation in *HCN2* causing an amino acid substitution in the S6 transmembrane segment (p.Gly460Asp). Whole-cell patch-clamp experiments were performed on primary culture of neonatal rat overexpressing the HCN2 WT plasmid, the mutated one or both to mimic the genetic background of the patient. Results confirmed what was previously found in HEK293 cells, thus a complete abolishment of the current in presence of the mutated channel alone and a significant reduction in the current density in the heterozygous condition. Also, the neuronal excitability was altered in presence of the mutation as the resting membrane potential resulted significantly depolarized. Immunofluorescence images suggested a trafficking impairment for the HCN2 mutated protein that seemed to accumulate in the perinuclear region. We conclude that the mutation p.Gly460Asp may cause a loss-of-function in HCN2 channel that could potentially affect the control of neuronal excitability.

Engineering of azobenzene-derived membrane-targeted photoswitches for light-driven modulation of neuronal activity

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Light-driven modulation of neuronal activity at high spatio-temporal resolution is becoming of high interest in neuroscience. Existing approaches, such as optogenetics, have shown very promising results, but concerns regarding its human applications are rising. As an alternative, we envision the use of azobenzene-based photoswitches targeted to the cell membrane. Azobenzene has the peculiarity to change reversibly from trans- into cis-conformation upon light stimulation. In a previous study, we demonstrated that the engineered light-sensitive azobenzene-based Ziapin2 successfully targets cell membranes and induces a biphasic hyperpolarization-to-depolarization membrane voltage modulation, triggering action potential firing in neurons in vitro and in vivo when stimulated with light pulses of appropriate wavelength. However, the main drawback of Ziapin2 is the transient permanence in the neuronal membrane that limits its long-term efficacy. Here, we developed and validated new generation of Ziapin-like molecules with more potent and persistent efficacy in neuronal photoexcitation by live imaging and electrophysiological recordings that can overcome, at least in part, the limitations of the original compound. In view of these features, these molecules display a high potential for future applications for visual restoration in photoreceptor degeneration, in the absence of genetic manipulations or implantation of externally powered retinal prostheses.

Effect of social choice-induced voluntary abstinence on incubation of methamphetamine craving and AMPA receptor expression in nucleus accumbens core

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Cue-induced cocaine and methamphetamine (Meth) craving progressively intensifies during forced abstinence from drug self-administration (a phenomenon termed incubation of craving). Previous evidence shows that incubation of craving after forced abstinence from psychostimulants in rats is associated with an accumulation of calcium-permeable AMPA receptors (CP-AMPARs) in the nucleus accumbens core. Conversely, social choice-based voluntary abstinence in rats attenuates the emergence of incubation of Meth craving. In the current study we performed whole-cell patch clamp recordings of AMPAR currents to investigate whether social choice-based voluntary abstinence prevents CP-AMPAR accumulation in the nucleus accumbens core, thereby reducing the incubation of Meth craving.

Synthetic torpor stimulates a latent physiological neuroprotective process able to cope with the brain accumulation of hyperphosphorylated tau protein

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Hyperphosphorylated tau protein (PPtau) represents the main pathophysiological marker of tauopathies, normally evolving to neurofibrillary tangles. Following “synthetic torpor” (ST), a condition very similar to natural torpor but pharmacologically induced on rats, PPtau accumulation is reversible, returning to normal levels within a few hours. Apparently, ST trigger a neuroprotective mechanism and the aim was to describe it. ST was induced on 15 male rats. Samples from parietal cortex and hippocampus were collected at these experimental conditions: C, control group; N, nadir of hypothermia; ER, early recovery, when animals reached normothermia following N; R3 and R6, 3h and 6h following ER, respectively. AT8 (p[S202/T205]-tau), Tau-1 (non-phosphorylated tau form), p[S9]-GSK3β (the inhibited form of the main kinase targeting tau), PP2A (the main phosphatase targeting tau), p[S473]-Akt (active anti-apoptotic factor) and clived-Caspase3 (active apoptotic factor) were determined by western-blot. Results were, in respect to C: N, high levels of AT8 and p[S9]-GSK3β, low levels of Tau-1; ER high level of p[S473]-Akt; low level of PP2A at R3. All factors returned normal at R6. No changes emerged for clived-Caspase3. Present data may suggest that ST elicits a neuroprotective physiological response, never described before, that could pave the way for a possible effective new strategy to contrast tauopathies, by pharmacologically stimulating this process at physiological temperature.

A failure of Amyloid-β physiological function due to deletions of α7 nicotinic acetylcholine receptors triggers an Alzheimer's disease-like pathology

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The increase of amyloid-beta peptide (Aβ) and the failure of cholinergic transmission are considered key events in Alzheimer's disease (AD) pathogenesis. However, previous works have demonstrated that Aβ at low concentrations acts through α7 nicotinic acetylcholine receptors (α7nAChRs) to ensure synaptic plasticity and memory in the healthy brain. Based on these findings, we hypothesized that α7nAChRs deletion might induce a compensatory increase of Aβ production that, in turn, would trigger an AD-like pathology. To validate this hypothesis, we studied α7nAChR Knock out (α7 KO) mice at different ages. We found that α7 KO mice presented an age-dependent impairment of synaptic plasticity and memory, starting at 12 months of age, paralleled by an increase of Aβ levels and Amyloid Precursor Protein expression. This was accompanied by hyperphosphorylation of tau at residues Ser 199, Ser 396, Thr 205, a decrease of GSK-3β phosphorylation at Ser9, an increase of PHF-1 immunoreactivity and the presence of paired helical filaments and neurofibrillary tangles. Finally, neuronal loss and increased GFAP-positive astrocytes were detected in hippocampi from 12-months-old α7 KO mice. Our findings suggest that α7nAChRs malfunction might precede the increase of Aβ and tau in AD pathogenesis, providing a different perspective to interpret how the failure of Aβ physiological function at the synapse might contribute to the disease.

Age-related changes in cardiac autonomic modulation and heart rate variability in mice

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In humans, the natural process of aging is associated with an enhanced susceptibility to arrhythmias, which may be caused by age-dependent modifications of cardiac autonomic nervous system (ANS) function. To understand the mechanism behind this impairment it is important to ascertain whether mouse model is an appropriate tool to study the cardiac ANS changes that characterize physiological aging in humans.
In this study, Heart Rate (HR) and Heart Rate Variability (HRV) parameters were extrapolated from ECGs recorded in two groups of freely-moving mice of different ages (4 and 19 month-old), in control conditions and after different ANS blocks. HR and HRV analysis revealed a decline of cardiac vagal modulation with age. Despite this, basal HR was unchanged in the two groups, since intrinsic HR was lower in the older mice. Both time- and frequency-domain HRV indexes were reduced following muscarinic, but not β-adrenergic block in younger mice, and to a lesser extent in older mice, suggesting that HRV is largely modulated by vagal tone in mice. Finally, older mice showed a larger vulnerability to spontaneous and isoprenaline-induced arrhythmias.
The present study combines HRV analysis and selective pharmacological ANS blockades to document an age-related impairment in cardiac vagal modulation in mice, which is consistent with the human condition. Furthermore, it reveals that HRV is a good analysis method to detect vagal but not sympathetic influences on cardiac function in mice.

Maternal exposure to very low dose of BPA induces alteration of mevalonate pathway in the liver and brain of rat fetuses

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Bisphenol A (BPA) is an organic chemical compound widely used for manufacturing plastics. BPA exposure originates principally from the diet, and it is detectable in over 90% of individuals, including pregnant women. Of particular concern is the effects of this exposure on the fetus, as it is a harmful endocrine disruptor related to metabolic and neurological diseases in later life. In fact, the mevalonate (MVA)/cholesterol metabolism plays a crucial role in whole-body functions, particularly in liver and brain. Thus, we aimed at investigating the impact of prenatal exposure to BPA in the liver and brain of rat fetuses from a sex-dependent point of view. Our results demonstrate that the liver of rat fetuses, *in utero* exposed to a very low dose of BPA (2.5 µg/kg/day), displayed significant changes of the proteins involved in cholesterol and fatty acid biosynthesis and trafficking. Moreover, an impact on inflammatory process was observed. The fetal brain exposed to BPA, displayed altered MVA pathway activation, increased protein prenylation, and a decreased level of pro-BDNF. Interestingly, all the effects in the brain were present in both sexes, while the modulations observed in fetal liver were dependent on sex, being noticeable only in female. In conclusion, this work demonstrates that maternal exposure to BPA compromises metabolic and Signaling Pathways very early in development and raises human health concerns about exposure to doses currently considered safe.

Involvement of TRPVs channels in the response to fluid osmolarity by rat diaphragmatic lymphatic vessels

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Lymphatic vessels intrinsic contractions allow lymph transport along collecting lymphatics and are modulated by stimuli including temperature and interstitial fluid osmolarity. In diaphragmatic lymphatics hyperosmolarity induces a monotonical reduction of contraction frequency (f_c), whereas an early increase and a late decrease in f_c can be seen in hyposmotic conditions. We investigated the receptors involved in such mechanisms, focusing on vanilloid transient receptor potential (TRPVs) channels.
Excised spontaneously contracting diaphragmatic lymphatics were challenged at 35°C using hypo and hyperosmotic conditions (290-324 mOsm) and different TRPVs inhibitors. Data show that in hyperosmotic conditions the not specific TRPVs blocker Ruthenium Red (RuR, 10-20 µM) reduced the osmo-dependent modulation of f_c in a dose-dependent manner while the specific TRPV1 inhibitor capsazepine (10µM) abolished the osmo-related f_c reduction. RuR application in hyposmotic solution affected both early and late f_c responses. Moreover, HC067047 (2.5-10 µM), a TRPV4 inhibitor, shifted f_c peak of ~2 minutes in a dose dependent manner not affecting the maximum f_c value. DCPBI, a volume regulated anion channels (VRACs) inhibitor, abolished the early f_c peak, whereas blocking TRPV1 decreased the late f_c reduction.
Overall, TRPV1 likely is the receptor involved in sensing hyperosmolarity, while a more complex mechanism involving at least TRPV1, TRPV4 and VRACs is related to hyposmotic response.

Does Angiotensin II contractile response undergo to age-related changes in rat jejunum?

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Renin-Angiotensin System was shown to modulate different functions in the gut,as motility. Age-related change in mRNA expression of Angiotensin (Ang II) receptor (ATR) in rat jejunum was reported. Thus, we aimed to characterize, *in vitro*, the contractile effect induced by AngII in jejunum from young (20-50 days old) and adult rats (> 1-year-old) to evaluate possible functional differences due to the change in receptor expression. Isometric tension *in vitro* was recorded in segments of jejunum and the response to Ang II alone or in the presence of ATR antagonists was analyzed. Action mechanism was also investigated. Ang II elicited a contraction in young and adult jejunum, reduced by Losartan, AT1R antagonist, and increased by PD123319, AT2R antagonist, as well as by the neural blocker, ω-conotoxin, or the NOS blocker, L-NAME. No difference was observed between young and old group. U-73122, phospholipase C inhibitor, or 2-aminoethoxy-diphenylborate (2-APB), IP_3 receptor inhibitor, or nifedipine, L-type calcium channel blocker, decreased AT1R mediated contractile response. To conclude, Ang II positively modulates the spontaneous contractile activity of rat jejunum via post-junctional AT1R and pre-junctional AT2R, located on the enteric nitrergic nerves. Activation of AT1R is multiphasic, involving Ca^{2+} mobilization from intracellular stores via PLC/IP_3 pathway and from extracellular space. No age related changes were observed in the potency or in the Ang II action mechanism.

Vasopressin downregulates the AQP3 function via V1aR in human colon HCT8 cells

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Vasopressin (AVP) plays a key function in controlling body water and salt balance through the activation of the vasopressin receptors V1aR and V2R. Beyond kidneys, the colonic epithelium modulates water and salt homeostasis. Abnormal secretion of AVP can cause the syndrome of inappropriate antidiuresis that leads to hyponatremia, which is an electrolyte disorder often observed in hospitalized and oncologic patients. Here, the action of vasopressin on the AQP3 was evaluated using human colon HCT8 cells as a model. Confocal and Western Blotting analysis revealed that HCT8 cells express both V1aR and V2R. Long-term (72h) treatment with dDAVP, a vasopressin agonist, reduces the membrane expression of AQP3, glycerol uptake, and cell viability. These effects were prevented by SR49059, a synthetic antagonist of V1aR, but not by tolvaptan, a specific V2R inhibitor. Of note, the SR49059 action was impaired by DFP00173, a selective inhibitor of AQP3. Interestingly, compared to the normal colonic mucosa, in colon of patients with adenocarcinoma, the expression of V1aR is significantly decreased with a partial increase in AQP3 expression. These findings were confirmed by gene expression analysis with RNA-Seq data. Overall, data suggest that AVP, through the V1aR dependent pathway, reduces AQP3 function, a process that is reversed in adenocarcinoma, suggesting that the AVP-dependent AQP3 pathway may represent a novel target in colon diseases associated with abnormal cell growth.

Environmental signals modify gut microbiome and metabolome enhancing neural plasticity through short-chain fatty acids

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Gut microorganisms and the products of their metabolism thoroughly affect host brain development, function and behavior. Since alterations of brain plasticity and cognition have been demonstrated upon motor, sensorial and social enrichment of the housing conditions, we hypothesized that gut microbiota and metabolome could be altered by environmental stimuli, providing part of the missing link among environmental signals and brain effects. Metagenomic and metabolomic analyses of mice housed in different environmental conditions, standard and enriched, identified environment-specific microbial community and metabolic profiles. We show that mice housed in an enriched environment have a reduction in gut bacterial richness and diversity indexes, and are characterized by a metabolomic fingerprint with the increase of formate and acetate and the decrease of bile salts. We demonstrate that mice treated with a mixture of formate and acetate recapitulate some of the brain plasticity effects induced by environmental enrichment, such as hippocampal neurogenesis, neurotrophin production and cognitive behaviors, that can be further exploited to decipher the mechanisms involved in experience-dependent brain plasticity.

The interaction of bile acids with dopamine transporter heterologously expressed in *Xenopus laevis* oocytes

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Several studies report the presence of bile acids (BAs) in the brain. Recently, it has been discovered in mice that bile diversion surgery increases circulating BAs, affects dopamine dynamics in the nucleus accumbens and reduces reward-related behaviour induced by cocaine. Feeding obeticholic acid (OCA), an FDA-approved semi-synthetic bile acid, to mice induced the same effects in the absence of surgery. These modifications *in vivo* were dependent on the presence of the plasma membrane Takeda G protein-coupled receptor 5 (TGR5). TGR5 is expressed in the intestine, as well as in astrocytes and neurons. Dopamine is a neurotransmitter involved in different physiological functions including reward. The dopamine re-uptake is mediated by the dopamine transporter (DAT). This work investigates the interaction between OCA and mDAT heterologously expressed in *Xenopus laevis* oocytes. The dopamine transport behaviour was studied by two electrodes voltage clamp. The data show that the OCA acts directly on mDAT, independently from the expression of TGR5. The binding of OCA with mDAT induces a small fast-inward current in sodium buffer and blocks the lithium leak current. Dose-response experiments in the presence of OCA resulted in unaltered I_{max} and $K_{0.5}$. Perfusing a different bile acid, lithocholic acid (LCA), induces similar behaviour of the transporter. These preliminary results indicate a novel and undocumented interaction between DAT (and potentially other NTTs) and BAs.

Regional activation within human rectus femoris muscle; physiological/biomechanical background and methodological applications

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Rectus femoris (RF) muscle is the bi-articular muscle contributing to both hip flexion and knee extension joint torques. Also, since strain injury during athletic events frequently occurs in RF muscle and pathological gait in neurological disorders is often explained by impaired activation of RF muscle, activation of RF muscle have been investigated in the research areas of human movements. Recent our studies using high-density surface electromyography and intramuscular electromyograms suggest that proximal and distal regions of RF muscle preferentially contribute to hip flexion and knee extension joint torque, respectively. For correct understandings of activation of RF muscle, this region-specific activation should be considered. We introduce its physiological/biomechanical background and methodological applications in this symposium.

Separate interneuronal pathways to control force and muscle co-contraction

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The central nervous system may produce the same endpoint trajectory or force with different muscle activation patterns. What differentiates these patterns is the presence of co-contraction, which does not contribute to force but allows to modulate stiffness. Whether co-contraction is controlled through the same interneuronal pathways involved in determining force is still unclear. We hypothesized the existence of a separate population of premotor interneurons providing shared drive to antagonist muscles, underpinning the control of co-contraction, independent of the interneurons underlying the control of force. We tested this hypothesis during an isometric task in which participants concurrently generated multi-directional forces and modulated the co-contraction of upper limb muscles to displace and stabilize a virtual end-effector. The spike trains of motor neurons were decomposed from high-density surface EMGs collected from two antagonist muscles, Biceps Brachii and Triceps Brachii, and the components of the neural input shared among muscles were identified through coherence analysis. We found a synchronization in the neural commands to antagonist muscles during co-contraction in the beta frequency band, i.e., in the band involved in transferring information but not in force generation, supporting the hypothesis of a separate interneuronal pathway for the modulation of co-contraction.

Physiological and technical insights gained into the assessment of muscle function from a combined approach: high-density surface EMG and electrical stimulation

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Traditionally, surface electromyograms (EMGs) have been collected with a single pair of electrodes. Of progressively broader interest is, however, the possibility of sampling EMGs with multiple electrodes. On one hand, such high-density recording provides a new set of information of physiological interest. On the other hand, it revealed the assessment of muscle function from surface EMG to be a grueling process. Most critically, users can only expect the amplitude of surface EMGs to be directly related to the degree of muscle excitation; the physiological validity of surface EMGs demands contending with the effect of factors of different nature. It is then that combining high-density recordings with electrical stimulation earn importance; some of the issues often affecting the relationship between surface EMG and muscle excitation may be controlled for in electrically elicited contractions. This presentation has a dual goal. First, evidence will be provided on how combining high-density EMG and electrical stimulation may contribute to advancing our knowledge on the acute, muscle adaptations to eccentric exercises. Second, the combined, high-density-stimulation approach will be used to highlight the importance of controlling for the effect of non-physiological sources before attempting to make claims on muscle function from surface EMG. It is our belief that only through broadening our physiological and technical knowledge can the potential of surface EMG be fully exploited.

Motor neuron synergistic organization in complex hand gestures

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Recently, how motor neurons innervating multiple muscles receive a net common synaptic input from spinal inter-neuronal integration to achieve synergistic muscular coordination across multiple tasks has started to be explored. So far, motor neuron synergistic organization has been studied only on a limited range of grips involving all the fingers, but a more extensive investigation involving a vast range of gestures is still to be provided.
Here, for the first time, we consider the motor neuron pools innervating 14 hand muscles, both extrinsic and intrinsic, by identifying averagely 93.4 ±13.8 motor units per subject among 5 subjects. Subjects were asked to achieve around 50 slightly different gestures such as single-finger flexion/extension for 3 wrist postures, 4 grip gestures and sign language letters. For each subject, we extracted motor neuron synergies by NMF on the smoothed spike trains of all the identified motor neurons. We also extracted motor neuron synergies by separating the contribution of extrinsic and intrinsic hand muscles. The identified synergies were then compared both in the three cases of muscle selection (all, extrinsic, intrinsic) and cross-correlated with hand kinematics. The clinical application of this neurophysiological framework can be in finding new biomarkers for rehabilitation or control signals for motor-neuron-based myoelectric control.

MicroRNA modulates cardiac electrophysiology via direct binding to ion channel

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Cardiac arrhythmias are a leading cause of morbidity and mortality. MicroRNAs (miRs) regulate the (electro)physiology of the heart and remodeling by well-recognized canonical RNAi mechanism. We recently discovered a novel new action for miRs that directly bind to and modulate the function of ion channel. We found that miR1, the most predominant miRs in the heart, physically binds with an inward rectifier K^+ channel Kir2.1, resulting in direct suppression of the inward rectifier potassium current (I_{K1}). This evolutionarily-conserved miR1-Kir2.1 interaction endogenously exists in cardiomyocytes, leading to physical modulation of cardiac cellular electrophysiology. We found that a human single nucleotide polymorphism (hSNP) of miR1, hSNP14A/G, is a mutant that specifically abolishes the biophysical action while maintaining the RNAi function of miR1, validating that the biophysical modulation is independent of RNAi. Significantly, miR1 but not hSNP14A/G eliminates the high inducibility of arrhythmia in miR1-deficient hearts. Our discoveries demonstrate that miRs modulate the electrophysiology of the heart through two different mechanisms: 1) conventional RNAi that regulates the expression of ion channel proteins, and 2) direct binding with ion channels that quickly results in electrophysiological modulation. Our study provides more comprehensive understanding of ion-channel dysregulation associated with cardiac arrhythmias.

Autoimmune channelopathies as a novel mechanism in cardiac arrhythmias

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Cardiac arrhythmias account for a considerable burden of morbidity and mortality for Sudden Cardiac Death (SCD) in industrialized countries. An underlying coronary artery disease and/or heart failure are the prevalent causes of cardiac arrest and SCD. However, in 5-15% of patients, structural abnormalities of the heart are absent at autopsy. In some of these subjects, mutations in genes encoding cardiac ion channels are documented, but the molecular autopsy is negative in nearly 70% of patients. Mounting evidence demontrates that autoimmunity is involved in the pathogenesis of cardiac arrhythmias and several arrhythmogenic autoantibodies targeting specific calcium, potassium, or sodium channels in the heart have been identified. Evidence exists that these autoantibodies can promote conduction disturbances and life-threatening tachyarrhythmias by inducing substantial electrophysiological changes. Thus, the term 'autoimmune cardiac channelopathies' has been proposed to define this novel pathogenic mechanism of cardiac arrhythmias, which could be more frequent and clinically relevant than currently acknowledged. Indeed, pathogenic autoantibodies against ion channels are detectable not only in patients with manifest autoimmune disease, but also in apparently healthy individuals, thus suggesting a causal role in some cases of unexplained arrhythmias and cardiac arrest/SCD.

Innate immune receptors, key actors in cardiovascular diseases

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Heart failure (HF) is a consequence of structural, neurohormonal, genetic, electric and functional impairment and, in many cases, is closely linked to left ventricle dysfunction. Recent clinical trial data implicate inflammation as a key player in cardiac HF-damage. An increase in the inflammatory response is mainly the result of sustained activation of the innate immune system, and several receptors of the innate immune system, such as nucleotide like receptors (NLRs), are increasingly recognized as new players in the progression of several cardiovascular diseases, including HF. Notable among these is NOD1 (nucleotide oligomerization domain type 1), an NLR member directly implicated in ventricular damage both in human and experimental HF mainly by impairing cardiac excitation-contraction coupling. Recent studies point out the innate immune system and cellular Ca^{2+} dynamics create a vicious cycle between Ca^{2+} sensing-Ca^{2+} mishandling and pro-inflammatory signaling that leads to cardiac dysfunction and finally to HF development. Herein, innate immune receptors stand as a new promising hub for new therapeutic targets for Ca^{2+} handling impairment in HF.

KNa1.1: a novel player in cardiac electrophysiology

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KNa1.1 is a sodium-activated potassium channel encoded by the *KCNT1* gene. Its role is well recognized in neuronal function where it should contribute to the prolongation of the late hyperpolarization and to the genesis of afterhyperpolarization, thus sustaining reduced neuronal excitability that protects cells from repetitive firing. KNa1.1 function in the cardiac tissue is less clear. The high intracellular sodium concentrations necessary to induce half-maximal channel activation conferred it a pathophysiological role in myocardial ischemia in mice, but its physiological contribution has yet to be identified. Recent data obtained in cardiomyocytes derived from human induced pluripotent cells correlates its expression with a reduction in action potential amplitude, in Vmax, and with a shortening of the action potential duration at 50 and 90% of the ripolarization. We confirm its expression in the adult human heart and suggest a preferential localization at the level of the intercalated disks. Moreover, a mutation in the KCNT1 gene with a pathogenic cardiac mechanism was found in two families affected by Brugada Syndrome and seizure. Taken all these elements as a whole, we propose KNa1.1 channel as a novel physiological modulators of the cardiac activity.

Neuromuscular electrical stimulation affects spinal excitability but not cortical activity of the somatosensory cortex

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Neuromuscular electrical stimulation (NMES) superimposed to voluntary muscle contraction has emerged as an innovative training and rehabilitation modality. However, its effects on spinal and central pathways are still unclear. The aim of this study was to investigate acute responses in spinal excitability, and in cortical activation of the somatosensory areas, following NMES superimposed to isometric contraction (NMES+), passive NMES (pNMES), and voluntary isometric contractions of the plantar-flexors (ISO).
NMES was delivered over the triceps surae muscle of ten young adults (age 28 ± 4 years). Fifteen intermittent contractions were performed at 20% MVIC of plantar-flexion torque. H-reflexes of the soleus were assessed via sEMG. EEG was recorded by 64 scalp electrodes. The amplitudes and latencies of the P40 and P100 SEP components evoked by the reflex stimulation were detected.
H-reflex amplitudes increased by 13% following NMES+ ($p<0.01$) decreased by 10% after pNMES ($p<0.05$). The amplitude and latency of P40 and P100 were unaffected by the three conditions ($p>0.05$).
Results indicated that NMES+ induces acute potentiation of the soleus H-reflex. This could reflect a combination of greater motor neuronal and spinal excitability. However, cortical activation of somatosensory areas remained unaltered. These findings provide novel information on the neurophysiological mechanisms underlying NMES+ which could be used to improve the effectiveness of traditional protocols.

Motor control of upper limb movement: functional significance of sensory overactivation and visual-proprioceptive interaction

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This study aimed to verify whether focal muscle vibration (fmv) may affect motor control of the upper limb movement and which sensorimotor arrangements may contribute to the purpose.
To answer this question, we vibrated the slightly tonically contracted anterior deltoid (AD), posterior deltoid (PD), and pectoralis major muscles in different combinations in healthy subjects at a frequency of 100 Hz for 10 minutes in single or repetitive administrations. We evaluated the vibration effect immediately after fmv and one week later on upper limb targeted movements tasks execute with or without visual feedback. We assessed target accuracy, movement mean, peak speed, and normalized Jerk using a 3D optoelectronic motion capture system. Further, we evaluated AD and PD activity during the tasks using wireless electromyography.
We found that fmv may induce increases in movement accuracy, mean speed and smoothness, and changes in electromyographic activity. However, the main effects were detected over time after repetitive vibration of AD and PD and when visual feedback was present before and during tasks execution.
Thus, in healthy subjects, fmv might affect the motor control of the upper limb movement over time, and optimized sensorimotor arrangements require visual-proprioceptive interaction to enhance this effect. Our finding implies that fmv in optimized stimulation/task execution conditions may improve the ability to promote expected motor outcomes and motor learning phenomena.

A novel viewpoint on anticipatory postural adjustments during gait initiation

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Anticipatory Postural Adjustments (APAs) are coordinated muscular activities aiming to counteract the postural perturbations produced by the voluntary movements. Many studies about gait initiation indicate as APAs those activities that precede heel-off of the leading foot, choosing the heel-off as the onset of voluntary movement. However, since initiating gait means pushing forward the body rather than lifting the foot, the onset of gait should be the displacement of the Centre of Mass (CoM), which starts with the backward shift of the Center of Pressure (CoP). Therefore, leg muscles driving such shift are prime movers and APAs should be searched for in trunk muscles.
In order to test this hypothesis, we analyzed gait initiation in 15 right-footed healthy subjects. The CoP position and the electromyographic activities were measured with a force plate and surface probes, respectively.
On the right side, Rectus and Obliquous Abdominis were active in 11 and 13 subjects, respectively, starting in average 33 and 54 ms before the CoP shift; Erector Spinae at L2 and T3 levels were instead inhibited (9 and 7 subjects, 104 and 120 ms). On the contralateral side, the same muscles showed excitatory APAs (Rectus and Obliquous Abdominis in 11 and 12 subjects, 27 and 82 ms; Erector Spinae in 10 and 7 subjects, 75 and 32 ms).
Present results provide a novel framework for distinguishing postural from voluntary actions, which may be relevant for gait disorders diagnosis and rehabilitation.

Calcium Entry Units: focus on the alternative door for calcium ions in skeletal muscle

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Store-operated Ca^{2+} entry (SOCE) is a mechanism, first described in non-excitable cells, that is triggered by depletion of intracellular Ca^{2+} stores (sarcoplasmic reticulum, SR). The two essential molecular players in SOCE are: a) STIM1, the Ca^{2+} sensor located in the SR, and b) Orai1, a Ca^{2+} permeable channel placed in the transverse tubules (TTs). SOCE in muscle has been proposed to be activated during muscle fatigue. However, which sites allow STIM1-Orai1 interaction in skeletal fibers has been long debated.
We recently discovered that exercise in mice drives formation of new junctions between stacks of SR cisternae and TTs which enhance STIM1-Orai1 interaction and entry of divalent cations. We proposed that these previously unidentified SR-TT junctions function as *Ca^{2+} Entry Units* (CEUs), providing a preferential pathway for rapid reuptake of Ca^{2+} into the SR during repetitive muscle activity. We also discovered that CEUs are dynamic junctions, as they increase in number and size during exercise, while disassemble following recovery.
We are now investigating whether CEUs can assemble *ex-vivo* in isolated muscles (in absence of innervation and blood supply) and which intracellular parameters may influence their assembly during exercise. Results collected indicates that a) CEUs can assemble in isolated muscles and that b) pH and temperature seems to have a strong effect on their formation.

Baroreflex and operating point dynamics at exercise onset in hypoxia

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At exercise onset, arterial baroreflex sensitivity (BRS) at the operating point (OP) decreases and the OP moves toward higher mean arterial pressure (MAP) and lower R-to-R interval (RRi) values. BRS modulation was ascribed to vagal withdrawal; the OP resetting to sympathetic activation. If this is so, BRS dynamics at exercise onset should differ when the vagal activity is low and the sympathetic activity is high, as in hypoxia (H).
To test this, ten healthy subjects performed three exercise bouts from rest (R) to 50W (E) on a cycle ergometer in normoxia (N) and H (O_2=11%). We recorded beat-by-beat MAP by finger cuff [mmHg] and RRi by ECG [ms]. At steady state, BRS [ms $mmHg^{-1}$] was computed with the sequence method. In the transient, the initial positive segment of the RRi vs MAP relationship was also treated as a sequence and its slope (S) was taken as indicative of BRS.
In N, BRS was 14.0±4.2 in R and 7.1±2.6 in E ($p<0.01$). At exercise onset, S was 8.1±4.8 ($p<0.01$ vs R; $p=0.93$ vs E). Afterward, MAP moved from 83±12 to 90±9 ($p<0.01$), and RRi from 770±114 to 626±84 ($p<0.01$). The new OP was reached in less than 10s.
In H, BRS was 10.7±2.9 in R ($p<0.05$ vs N) and 2.9±1.5 in E ($p<0.01$ vs R and N). At exercise onset, S was 5.6±2.6 ($p<0.01$ vs R; $p<0.05$ vs E). Then, MAP moved from 83±12 to 89±14 ($p<0.05$), and RRi from 712±71 ($p<0.01$ vs N) to 497±71 ($p<0.01$ vs rest and N). The new OP was reached in more than 50s.
H showed a slower BRS decrease and OP shifting at exercise onset than N.

Poster Abstracts

(presenting authors are shown underlined)

P1.1

Radiotherapy-induced effects on microglia support glioblastoma growth and malignance by tumor microenvironment alteration

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Tumor microenvironment (TME) consists in a complex interplay of cells and soluble factors holding a critical role in neoplastic development. GBM, a WHO grade IV glioma, is a malignant primary brain tumor for which combination of surgery, chemotherapy and radiotherapy is the first-line approach despite severe adverse effects. Significant pathophysiological changes have been found in GBM TME, such as oxidative stress, neuroinflammation and glia activation. This has been reported to occur spontaneously and upon severe therapeutic regimens, resulting in dismal prognosis and recurrences. Microglia, is among the most important players in favouring GBM growth and proliferation, representing target cells of immune escape mechanisms. Our study aims at analysing direct and indirect effects mediated by irradiation in modulating intercellular communication. We first evaluated the radiation-induced effects on the microglia and cell-to-cell communication mediated by both paracrine and autocrine interactions in order to understand the molecular mechanisms involved in immunosuppression and tumor progression and growth. Conditioned media from irradiated GBM and microglia cell lines were collected to evaluate effects on U-87 MG and U-251 MG, highlighting significant effects on apoptosis, DNA damage and proliferation. Our results suggest that radiotherapy modulates microglia to sustain and promote GBM neoplastic growth and malignance, inducing an immunosuppressive TME.

P1.2

Atrial-like cardiomyocytes derived from human pluripotent stem cells: *in vitro* modeling of atrial cardiomyopathies

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Background: Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) are an attractive source for disease modeling and pre-clinical drug testing. However, standard cardiac differentiation protocols result in a mixed population of ventricular-, atrial- and nodal-like cells, limiting the reliability for studying mechanisms of atrial fibrillation. We applied retinoic acid (RA), known to induce atrial phenotype. We aim to develop a hiPSC-based *in vitro* platform, for modeling human atrial-specific cardiomyopathies.
Methods and Results: iPSCs-CMs were differentiated toward atrial-like phenotype by applying 1µM RA, in parallel with the conventional protocol (Ctrl). Contraction profiles of RA CMs, recorded by MuscleMotion algorithm, yielded higher beating frequency with shorter duration, time to peak, and relaxation time. Consistently, extracellular field potentials (FP) recorded by Multi Electrode Array (MEA) were shorter in RA-treated CMs than in Ctrl one. Patch-clamp was useful to identify atrial *vs* ventricular action potential parameters by injecting an appropriate I_{K1} computational model. RT-qPCR and IC confirmed a higher percentage of atrial-like cells in RA-CMs by overexpression of atrial markers in RA, and downregulation of ventricular ones.
Conclusions: our human *in vitro* model can be a reliable platform to study the mechanism underlying inherited or induced atrial arrhythmia in human CMs, suitable to screen anti-arrhythmic agents in a translational approach.

P1.3

The long-lasting istaroxime metabolite PST3093 stimulates SERCA2a and reverses disease-induced changes in cardiac function

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Heart failure (HF) therapeutic toolkit would strongly benefit from the availability of ino-lusitropic agents with a favorable pharmacodynamics and safety profile. Istaroxime is an agent, combining Na^+/K^+ pump inhibition and SERCA2a stimulation, shown by phase 2 trials to be promising in the acute setting. As PST3093 is an istaroxime metabolite reaching plasma concentration and duration greater than those of istaroxime, its evaluation is crucial to establish its contribution to in HF therapy. We studied PST3093 for its effects on SERCA2a and Na^+/K^+ ATPase activities, Ca^{2+} dynamics in cardiomyocytes and hemodynamic effects in an in-vivo rat model of diabetic (streptozotocin (STZ)-induced) cardiomyopathy. The results converge to identify PST3093 as a “selective” (i.e. devoid of Na^+/K^+ pump inhibition) SERCA2a activator and in in-vivo echocardiographic assessment, PST3093 improved overall cardiac performance, reverting many of STZ-induced abnormalities. For i.v. administration, PST3093 toxicity was considerably lower than that of istaroxime and its evaluation against a panel of 50 targets commonly involved in cardiac and extracardiac side-effects, failed to reveal significant interactions. PST3093 is a “selective” SERCA2a activator, the prototype of a novel pharmacodynamic category with a potential in the ino-lusitropic approach to HF therapy that may contribute to the clinical efficacy of istaroxime infusion.

P1.4

Acidic microenvironment promotes Pancreatic Ductal Adenocarcinoma (PDAC) cells selection inducing more aggressive cancer cells: role of Store-Operated Ca^{2+} signals

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PDAC is characterized by an acidic microenvironment, that may promote its progression by selecting aggressive cancer cells. Aberrant Ca^{2+} signals are involved in cancer progression and pH-sensitive Ca^{2+}-permeable channels act as pH sensors, transducing signals to activate intracellular downstream pathways involved in PDAC progression. To study the role of acidic pHe in PDAC hallmarks and its interplay with Ca^{2+} signals, we compared different pH selection models for PANC1 cells. PANC1 cell proliferation, viability, cell adhesion and invasive abilities are impaired in 4 days pHe 6.6 cells respect to control cells, while low pH-selected cells for 1 month show higher proliferation rate, migration, adhesion and invasion respect to control cells. These cell activities closely correlate with Ca^{2+} signals, showing that pH-selected cells are characterized by an increased Store Operated Calcium Entry (SOCE) and faster intracellular ORAI1-dependent Ca^{2+} oscillations respect to control, with overexpression of ORAI1, a SOC channel, while 4 days pH 6.6 cells show decreased SOCE and ORAI1 expression. In conclusion, low pHe exposition decreases SOCE and slows Ca^{2+} oscillations, promoting apoptosis of weaker cancer cells, selecting more aggressive cancer cell phenotypes; in turn higher Ca^{2+} entry by upregulation of SOC channels and faster Ca^{2+} oscillations may trigger Ca^{2+}-dependent signaling pathways involved in PDAC progression.

P1.5

Characterization of chromatin architecture during malignant transformation of neuroblastoma cells by optical nanoscopy

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Despite its apparent disorder, chromatin organization is a well-orchestrated mechanism involved in cellular physiology and onset of many diseases, including cancer. Among these, Neuroblastoma (NB) is the most common extracranial solid tumor in childhood. We aim to assess possible changes in chromatin nanoscale architecture, possibly correlated with NB cell malignancy. The malignant cell line SKNBE2 is genetically engineered to overexpress the ncRNA NDM29 capable of differentiating the NB cells into a neuronal lineage. In both malignant NB cells (Mock) and neuron-like cells (S1.1), we explored chromatin by optical microscopy using both confocal microscope and stimulation emission depletion (STED) microscope. Through confocal microscopy, we assessed the nuclear 3D morphology of Mock *vs* S1.1 cells by measuring volume, shape, circularity, and chromatin compaction. Whilst with STED microscopy and histone H3 immunolabeling (H3K9 acetylation and H3K9 trimethylation) we characterized chromatin regions typically associated with highly and poorly condensed chromatin. Our preliminary data show differences in the nuclear morphometry, in the spatial arrangement of domains rich in acetylated or methylated H3K9, and chromatin compaction between Mock and S1.1 cells. These findings unveil differences in chromatin organization during malignant transformation, paving the way towards new prognostic approaches and understanding chromatin remodeling in physiological/pathological processes.

P1.6

Morpho-functional evidence of inflammatory sensitivity acquisition by Caco-2 cell monolayers after spontaneous differentiation towards the epithelial enterocyte-like cell phenotype

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In the gastrointestinal tract, severe pathological conditions, including those related to the inflammatory bowel disease (IBD), trigger inflammation processes, that affect the epithelial barrier and are poorly reversible. The enterocyte monolayer is a direct target of such processes inasmuch its barrier functionality is altered. Among early onsets, the monolayer undergoes cytoskeletal and more general morpho-functional rearrangements, including activation of certain immune gene pathways.
Here, we show the impact on intestinal cells of dextran sulfate sodium (DSS), used to mimic IBD damage in mice *in vivo*. To this aim, we used Caco-2 cells monolayers at two different stages of spontaneous differentiation, i.e. undifferentiated cells at 7 days post seeding (dps), and differentiated ("enterocyte-like") cells at 21 dps. Comparing monolayers at 7 *vs.* 21 dps, we detected differential dose- and stage-specific variations in morphometry and morphology, in association with transcriptional variations of immune/inflammation genes (IL1B, IL6, NFkB1, IFNG) or genes related to cell death pathways (TP53, CASP3).
Compared to the undifferentiated monolayer at 7 dps, we describe specific rearrangements, in terms of morphometry and gene expression, of the "enterocyte-like" monolayer at 21 dps which increases its physiological responsiveness to proinflammatory stimuli. Our approach helps to highlight how inflammatory sensitivity is an acquired trait of the maturation of the monolayer itself.

P1.7

iPSC-derived cardiomyocytes as a model to dissect electrical and mechanical dysfunctions of caveolinopathies

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Mutations in Caveolin-3 (CAV3), the key structural component of muscular caveolae, lead to some rare forms of hereditary skeletal myopathies and/or cardiomyopathies called caveolinopathies. We focus on the specific heterozygous mutation T78K, found in a patient with Rippling muscle disease and hyperCKemia (HCK). We have characterized human cardiomyocytes (CM) differentiated from induced pluripotent stem cells (iPSC) derived from this patient and from a healthy control. Since HCK implies muscle fibre breakdown, we have carried out osmotic shock experiments and found a significantly increased membrane fragility in T78K-CM. Patch-clamp analysis revealed an increased spontaneous beating rate of T78K-CM compared to CTRL and a significant shortening of action potential duration. In agreement with APD shortening, we recorded a larger transient outward potassium current.
Proteomic analysis also revealed alteration in calcium handling and sarcomeric proteins. For this reason, we analyzed the contraction properties using the FLEXcyte96 and CardioExcyte96 platforms in impedance recording mode (Nanion Technologies), thereby finding larger changes in mean beat amplitude (22.3 ±2.7 vs 11.5±0.9 Ohms at 1Hz), with faster rising and falling kinetics in T78K-CM compared to CTRL. Lastly, we analyzed the same parameters after pacing CMs at different rates. This revealed the inability of T78K-CM to adapt contraction to increasing rates, suggesting a possible E-C coupling dysfunction.

P1.8

Ablation of Aquaporin-9 improves the systemic inflammation of LPS-induced endotoxic shock in mouse

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Septic shock is the most severe complication of sepsis, characterized by a systemic inflammatory response, leading to multiple organ failure and dramatically high mortality. Aquaporin-9 (AQP9), a membrane channel expressed mainly in liver and leukocytes, has been associated with the inflammatory response. After showing AQP9 involvement in murine bone marrow DCs maturation and inflammatory cytokines release, here we evaluated whether AQP9 has a role in mouse systemic inflammation during endotoxic shock.
Wild type (WT) and *Aqp9* KO (*Aqp9*$^{-/-}$) male mice were submitted to endotoxic shock (i.p. LPS, 40 mg/kg) and the related survival times were followed during 72 h. Nitric oxide (NO) and superoxide anion (O_2^-) production and the expression of inducible NO-synthase (iNOS) and cyclooxigenase-2 (COX-2) were evaluated in various organs.
LPS-treated KO mice survived significantly longer than WT mice, and 25% of the KO mice fully recovered from the endotoxin treatment. The LPS-injected KO mice showed lower inflammatory NO and O_2^- production and reduced iNOS and COX-2 levels through impaired NF-κB p65 activation in liver, kidney, aorta and heart compared to the LPS-treated WT mice. Treatment of a rodent hepatoma cell line with HTS13286, an AQP9 blocker, prevented the LPS-induced increase of inflammatory NO and O_2^-.
A role for AQP9 in the early acute phase of LPS-induced endotoxic shock involving the NF-κB pathway is suggested. AQP9 modulation may be of translational value to treat sepsis.

P1.9

Gabapentin as a novel treatment for ocular pain

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The management of the ocular pain is currently limited to the use of palliative treatments, such as lubricants and anti-inflammatory drugs, that are available to alleviate patients' discomfort. Anesthetic drugs are not indicated, because they may interfere with the neural feedback between the cornea and the lacrimal gland, therefore impairing tear production and lacrimation. Gabapentin (GBT), an analogue of gamma-amino butyric acid, acts as a good pain reliefer following systemic administration in glaucomatous patients. In dry eye disease (DED), GBT has been demonstrated as a promising anti-inflammatory drug when topically administered. In this study, we investigated whether GBT given topically as eye drops has analgesic but not anesthetic effects using a rabbit model of formaldehyde-induced ocular pain. We found that, conversely to the anesthetic oxybuprocaine, topical GBT decreases corneal sensitivity without impairing the lacrimation, which resulted even stimulated. In the lacrimal gland, mechanisms underlying GBT-induced lacrimation included the activation of the autonomic nervous system with increased production of both acetylcholine and norepinephrine and the overexpression of aquaporin 5. In conclusion, anti-inflammatory, analgesic and secretagogue properties make GBT a promising candidate for the treatment of ocular pain such as in DED.

P1.10

Development of 3D-organotypic cultures for the analysis of NHE1 in epithelial remodeling during pancreatic morphogenesis

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Pancreatic epithelial ductal cells form branched tubes that, during digestion, secrete an alkaline, HCO_3 rich juice to neutralize stomach acidity. This leads to a parallel decrease of extracellular pH (pHe) in the interstitial extracellular matrix (ECM), which is translated into specular variations of intracellular pH (pHi). However, while the pancreas cyclically undergoes these acid-base fluxes, neither the effect of the changing pHe nor the role of one of the major pHi regulators, the Na^+/H^+ exchanger isoform 1, NHE1, on branching morphogenesis have been studied in a cell culture model physiomimetic of the human pancreas. 3D organotypic culture is a translational bridge between 2D cultures and animal models and has been used to study branching dynamics in other tissues. Here, we have established a 3D organotypic system for modeling normal human, pancreatic ductal architecture and analyze the role of NHE1 and pHe on branching morphogenetic dynamics. We found that when grown on hydrogels mimicking the natural pancreatic ECM, the cells create arborized epithelial ductal networks and their morphogenesis is affected by both NHE1 activity and pHe. Indeed, both NHE1 inhibition with its inhibitor, Cariporide, and pHe acidification from 7.4 to 6.7 resulted in a hyper-complex and stabile branching tubular network.
We propose an important role for NHE1 activity and pHe in shaping pancreatic ductal branching morphogenesis in line with the complex acid-base dynamics of this organ.

P1.11

Type 2 deiodinase-mediated intracellular activation of Thyroid Hormone promotes myogenic differentiation by regulating mitochondrial reactive oxygen species generation

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Thyroid hormone (TH) is a key metabolic regulator that coordinates short-term and long-term energy needs. The role of TH in regulating metabolic pathways includes a fine modulation of mitochondrial biogenesis and dynamics. However, the net effects on cellular respiration and generation of reactive oxygen species (ROS) remain unclear. We generated a doxycycline-inducible cell line, in which the expression of the TH-activating enzyme, type II deiodinase (D2), is reversibly turned on, as a cellular model of muscle hyperthyroidism. Interestingly, the intracellular activation of TH resulted in a net shift from oxidative phosphorylation to glycolysis with a consequent increase in extracellular acidification rate. As a result, both the basal and the doxorubicin-induced cellular ROS production was reduced. Importantly, the expression of the mitochondrial scavenger SOD2 was specifically induced at transcriptional level, by D2-mediated TH activation. Finally, we observed that the increased levels of SOD2 induced by D2 and the consequent attenuation of the oxidative stress were functional for a proper differentiating cascade of muscle cells, by evidencing that TH-SOD2 axis is essential for the myogenesis program trigged by D2 and TH. In conclusion, our findings indicate that the maintenance of physiological TH concentrations play a key role in regulation of ROS homeostasis and of oxidative stress and sheds new light on metabolic TH action relevant for muscle physiology.

P1.12

Exposure to Atrazine worsens bleomycin-induced pulmonary fibrosis by Nrf-2 pathways and induces behavioral alterations

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Background: pulmonary fibrosis can be caused by genetic abnormalities, autoimmune disorders or exposure to environmental pollutants. chemical name of ATR is 6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine, and it is the most commonly used broad-spectrum herbicide in agricultural crops. Some of the most accredited hypotheses that would explain the mechanism of toxicity induced by ATR is the production of reactive oxygen species (ROS) that lead to an unbalance in the physiological anti-oxidant system. But until today none investigated the impact of ATR exposure during pulmonary fibrosis. Methods: mice were subject or to ATR exposure, or to bleomycin injection or both. At the end of experiment lung and blood were collected; Results: Following ATR or Bleomycin induction we found a significantly increase in lung damage, fibrosis, and oxidative stress. This condition was significantly worsened when the animals injected with bleomycin were also exposed to ATR. Additionally, we found a significantly motor and non-motor impairment in animals exposed to ATR; Conclusions: Our study demonstrate that ATR exposure was in grade, through the inhibition of Nrf2 pathways to induce a significantly onset of oxidative stress condition that probably affect also the brain.

P1.13

Knock out of the GNB5 gene associated with the IDDCA syndrome alters the intrinsic and extrinsic rate of sinoatrial-like cells

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IDDCA is an autosomal recessive syndrome, characterized by global developmental delay and cardiac abnormalities, most commonly sick sinus syndrome (SSS) with bradycardia. The disease is characterized by loss of function mutations in *GNB5*, that encode for the G protein subunit β5. To study the *GNB5* role in development and function of sinus node cells (SAN), we used two lines of mouse embryonic stem cells (mESC) one *wild-type* and one *GNB5* knock-out. mESC were differentiated into cardiomyocytes using hanging drops method and CD166+ SAN precursors sorted at day 8 of differentiation. The proportion of CD166+ precursors was similar, and we did not observe any significant difference in the SAN developmental markers *Shox2* and *Tbx18*, suggesting that *GNB5* is not required for SAN development. We found *Hcn4* more expressed in KO cells while *TnnI3* and *Myh7* were less expressed in KO than in WT cells. Patch clamp analysis revealed that on average KO SAN-like cells showed a higher intrinsic rate (2.5±0.1 Hz) than WT cells (1.5±0.04 Hz) and displayed irregular pattern with pauses. We treated cells with isoproterenol (1μM) and carbachol (100nM), to mimic the stimulation of the autonomous nervous system. We found that both lines responded similarly to isoproterenol: carbachol induced a mild effect on WT cells while stopped the spontaneous activity of KO cell. Further analysis is necessary to elucidate the detailed molecular pathways beneath the cardiac manifestation caused by *GNB5* dysfunction.

P1.14

Sonoporation induces specific internalization in tumor cells leading to a temporary alteration of the vascularization

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Sonoporation, typically by means of ultrasonic frequencies, may be used to change the permeability of the cell membranes. By facilitating the internalization of siRNA, mRNA or drug molecules, it could have great potential to deliver non-permeable particles into the cytosol. Moreover, cellular stress, altered vascularization and tumor microenvironment modulation may contribute to the beneficial consequences of sonoporation. In our studies, we used unfocused ultrasound sonoporation, creating harmonic waves that interfere with the resonance frequency of cell membranes. We comparatively treated glioblastoma cells, pancreatic, thyroid and breast cancer cells with sonoporation and investigated the uptake of membrane impermeable dye. We first analysed the optimal value of modulation for each cell line to identify specific expositions able to selectively permeabilize cell membranes. We observed that a single modulation was able to increase significantly the permeability of U87 cells selectively, thus indicating that specific modulation can be used to target specific cell populations. Using a photoacoustic system by "VisualSonics Fujifilm", we moved to study in an in vivo model of glioblastoma, the effects of sonoporation, detecting a temporary increase in vascularization and oxygenation in the tumor. Therefore, the modifications of the pathophysiological parameters induced by sonoporation could be useful to develop new highly selective therapeutic strategies for tumors.

P1.15

Omega 3 effect on cell viability and mitochondrial dynamics: a focus on fusion/fission balance into liver cancer cells treated with EPA, DHA and capecitabine

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Omega-3s represent a potential adjuvant in chemotherapy as they could improve the therapeutic outcome. Present work aimed to evaluate capecitabine (a prodrug converted in the active metabolite 5-fluorouracil) effects, alone or in combination with the omega-3s eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on cell viability, mitochondrial dynamics (fusion/fission ratio), antioxidant defence and lipid accumulation in HepG2 cells. EPA and DHA alone led to an increase in Mfn2 (fusion marker) and a decrease in DRP1 (fission marker) and DRP1/MFN2 ratio without change in cell viability. Capecitabine induced cell viability reduction associated with a slight Mfn2 content increase. Noteworthy, capecitabine in combination with EPA and DHA induced a further reduction in cell viability, but in association with DRP1 and DRP1/MFN2 increases, suggesting a shift towards mitochondrial fission processes. SOD2 and GPx1 levels decreased in cell simultaneously treated with capecitabine, EPA and DHA, suggesting a reduction in antioxidant activity . In conclusion, EPA and DHA alone showed a positive effect on mitochondrial dynamics, whereas capecitabine exerted its antitumour activity by reducing cell viability in association with an adaptive increase in mitochondrial fusion. Omega3 and capecitabine combination increased capecitabine antitumour activity by further reducing cell viability in association with a shift toward fission processes and impairment in antioxidant defences.

P1.16

Hydrogen peroxide induces extracellular Ca^{2+} entry and apoptosis via transient receptor potential ankyrin 1 (TRPA1) channel activation in primary culture of metastatic colorectal carcinoma

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Reactive oxygen species (ROS) are a crucial component of tumour microenvironment that might promote cancer cell apoptosis. Transient receptor potential ankyrin 1 (TRPA1) is a polymodal non-selective cation channel that displays high permeability to Ca^{2+} and that may sense ROS, including hydrogen peroxide (H_2O_2), in several types of cancer. Herein, we sought to evaluate for the first time whether TRPA1 is expressed and serves as a redox sensor in primary culture of metastatic colorectal carcinoma (mCRC). We found that TRPA1 protein is expressed in mCRC cells, whereas allyl isothiocyanate (AITC), a selective TRPA1 agonist, induced larger extracellular Ca^{2+} influx in mCRC cells as compared to non-neoplastic cells. In accord, AITC-evoked Ca^{2+} entry was impaired by HC-030031, a selective TRPA1 antagonist, and by removing extracellular Ca^{2+}. Similarly, exogenous administration of H_2O_2 induced an increase in intracellular Ca^{2+}concentration, which was again larger in mCRC cells as respect to non neo-plastic cells and was abolished by blocking TRPA1 with HC-030031 and removing extracellular Ca^{2+}. Finally, AITC and H_2O_2 were found to engage caspase 3/7 pathway to induce apoptosis and reduce mCRC cell viability upon TRPA1 stimulation. These data support the notion that TRPA1 activation by AITC-containing dietary vegetables could represent an alternative therapeutic strategy to selectively sensitize mCRC, but not non-neoplastic, cells to oxidative stress.

P1.17

Mitochondrial changes in the cardiac response to hypoxia of the goldfish *Carassius auratus*

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The ability to tolerate periods of hypoxia differs among vertebrates. Several fish species survive and remain active for long times under low oxygen also thanks to important cardiovascular adaptations. In this study we used a natural model of hypoxia-tolerance, the goldfish *Carassius auratus,* to investigate the mechanisms that, by regulating mitochondrial biogenesis, preserve the cardiac function under prolonged hypoxia.
TEM analysis revealed that, compared to normoxic animals, the ventricle of goldfish exposed to 4 days of hypoxia (30% O_2) showed an extended mitochondrial compartment. This is paralleled by a modulation of the mtDNA/nDNA ratio, and of proteins involved in mitochondria dynamics. Western Blotting analyses revealed an increased expression of pro-fission markers, i.e., the mitochondrial dynamin-related protein 1 (Drp1) and the metalloendopeptidase 1 (OMA1), and a modulation of proteins involved in the mitochondrial fusion/fission equilibrium (i.e. the dynamin-related GTPase OPA1). This is also confirmed by immunolocalization analyses.
Overall, these data suggest that under protracted hypoxia the preserved cardiac function typical of the goldfish may increase mitochondrial content, and this allows to maintain an adequate energy supply for the beating heart.

P1.18

Mechanotransduction boosts the pancreatic β-cell function by inducing a mitomorphosis program

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Pancreatic β-cells are constantly exposed to mechanical forces arising from the extracellular environment, but how these stimuli affect the pancreatic β-cell function is poorly understood. By exploiting cluster-assembled zirconia substrates with controlled topography, we have recently demonstrated that β-cells sense and respond to nanoscale features of the microenvironment by activating a mechanotransductive pathway, which induces a reorganization of the cytoskeleton and improves the β-cell function. Since mitochondria are connected to the cytoskeleton and play a key role in regulating the β-cell activity, we evaluated whether the extracellular nanotopography may impact on mitochondrial homeostasis.
Morphological and functional studies demonstrated that the nanotopography evokes a mitomorphosis program that affects mitochondrial morphology and membrane potential. Accordingly, the proteomic analysis performed on the mitochondrial fraction revealed that the nanotopography modulated the expression of proteins involved in the mitochondrial respiratory chain, in cristae shaping and mitochondrial dynamics. Proteomic and functional analyses also showed changes in the expression of proteins shared by the endoplasmic reticulum (ER) and mitochondria (VDAC, HSP) and the reorganization of the mitochondrial-ER contact sites.
Our data suggest that the microenvironment through mechanotransductive pathways can control mitochondrial homeostasis and shape β-cell function accordingly.

P1.19

Proinflammatory cytokines as emerging molecular determinants in cardiolaminopathies

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Mutations in Lamin A/C gene (*lmna*) cause a wide spectrum of cardiolaminopathies strictly associated with significant deterioration of the electrical and contractile function of the heart. Fibrofatty replacement of the cardiac tissue is frequent because of myocardial inflammation, further aggravating the progression of the disease. Despite the continuous flow of biomedical evidence, linking cardiac inflammation to heart remodeling in patients harboring *LMNA* mutations is puzzling. Therefore, we profiled 27 serum cytokines/chemokines in patients belonging to four different families carrying pathogenic *lmna* mutations segregating with cardiac phenotypes at different stage of severity (n=19) and in healthy subjects (n=11). Regardless *lmna* mutation subtype, high levels of circulating G-CSF and IL-6 were found in all affected patients' sera. In addition, elevated levels of IL-1Ra, IL-1b IL-4, IL-5 and IL-8 and GM-CSF were measured in a large subset of patients associated with more aggressive clinical manifestations. Finally, the expression of the proinflammatory 70 kDa heat shock protein (Hsp70) was significantly increased in serum exosomes of patients harboring the *lmna* mutation associated with the more severe phenotype. Overall, the identification of patient subsets with overactive or dysregulated myocardial inflammatory responses could represent an innovative diagnostic, prognostic, and therapeutic tool against Lamin A/C cardiomyopathies.

P1.20

Effects of *in vivo* treatment with the novel mitochondria-targeted peptide elamipretide on lipid profile of cardiac mitochondria in a murine model of Barth Syndrome cardiomyopathy

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Barth Syndrome (BTHS) is a rare X-linked disease presenting cardiomyopathy, skeletal muscle weakness, fatigue, neutropenia, and abnormal growth. The disease is characterized by abnormalities of the phospholipid cardiolipin (CL), due to mutations in the tafazzin (TAZ) gene, responsible for CL remodelling in the inner mitochondrial membrane. Biochemical abnormalities in BTHS patients include a decreased level of mature CL and an increased level of monolysocardiolipin (MLCL) and dilysocardiolipin (DLCL).
One of the most recent promising therapeutic approach includes elamipretide, which is able to promote mitochondrial respiration and ATP production by interacting with CL, but mechanistic details of its pharmacological effects are still unknown.
In this study, we utilized a tetracycline inducible shRNA-mediated TAZ KD mouse model of BTHS to investigate whether the treatment with elamipretide could affect the lipid alterations in cardiac mitochondria.
The CL fingerprinting of isolated mitochondria have been obtained by MALDI-TOF/MS. Alteration in the MLCL/CL ratio between the mitochondrial samples has been used as a therapeutic monitoring of drug treatment. Furthermore, we have analysed for the first time lipids tightly bound to the electro-eluted OXPHOS complexes isolated from BNGE of mitochondria of TAZ KD mice treated or not with elemipretide. MALDI mass spectra clearly show the signals diagnostic for the presence of DLCL and MLCL in all OXPHOS complexes.

P1.21

Oxygen-dependent expression of beta-adrenoceptors and members of the HIF-1/VEGF axis in human Müller and retinal endothelial cells

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Retinopathy of prematurity (ROP) is a disease characterized by a first phase of hyperoxia and a second one of hypoxia. Hypoxia leads to neovascularization through an increase in the levels of hypoxia-inducible factor (HIF) 1 and its target vascular endothelial growth factor (VEGF). In hypoxic conditions, VEGF, mainly produced by Müller cells, stimulates endothelial cell proliferation acting at its receptor VEGFR2. An involvement of β-adrenoceptors (BARs) in VEGF production and retinal neovascularization has been assessed. Here, using human Müller and retinal endothelial cells (MIO-M1 and HREC, respectively) we evaluated for the first time the effects of hyperoxia (75% oxygen) and hypoxia (1% oxygen) on the expression of BARs, HIF-1, VEGF and VEGFR2.
Hyperoxia did not affect BAR1 and BAR2, whereas decreased BAR3 in both MIO-M1 and HREC. Hypoxia, instead, upregulated both BAR1 and BAR3 in MIO-M1 and HREC, having no effect on BAR2. In both cell lines, HIF-1 and VEGF were increased by hypoxia. In HREC, VEGFR2 was decreased by hyperoxia while it was not influenced by hypoxia; in MIO-M1, VEGFR2 was upregulated by hyperoxia and downregulated by hypoxia.
Although a correlation between BARs levels and the activity of the HIF-1/VEGF axis under hyperoxia/hypoxia is still to be demonstrated, the present results lay the ground for assessing whether targeting BARs during the different phases of ROP may be a strategy to avoid the development of neovascularization.

P1.22

Training-dependent effect of exosomes on skeletal muscle regeneration

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In human, the progressive loss of skeletal muscle mass and strength due to aging is called sarcopenia; one of the causes is the activity of satellite cells (SCs). SCs are muscle stem cells normally quiescent able to become activated, proliferate and differentiate to repair the damaged muscle fibre. Both fibers and SCs are important sites for the release of nanovesicles including exosomes (EXOs), suggesting that skeletal muscle plays the role of a secretory organ. The EXOs can represent a way of communication between close cells but even between distant cells since they can also be delivered by the circulation playing an important role of cross-talking. Recent studies suggest that proper physical activity slow down sarcopenia progression. It is known that following exercise, the muscle releases factors that can be conveyed through EXOs. The aim of the present study is to elucidate mechanisms that influence the difficulty of muscle regeneration in the elderly, investigating the EXOs released by myoblasts and myotubes pre- and post-training and those present at the systemic level. Our results show that miRNAs transported by the EXOs, modify their expression according to the type of training that is carried out, showing a different expression of miRNA in subjects trained with resistance protocol compared to endurance or sedentary subjects. The miRNAs released with the EXOs following resistance training appear to be correlated with positive effects on muscle regeneration.

P1.23

Effects on protein phosphorylation during postmortem: biomedical and forensic implications

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During postmortem phenomena, the proteoma undergo cumulative decay over the time resulting in a decrease in the range and abundance of tissue proteins as well as in an increase of post-translational modifications. The most important post-translational protein modification is the phosphorylation, which influences its biological activity, subcellular localization and interaction with other biological molecules. Postmortem tissues are extensively used in both biomedical and forensic studies, however postmortem protein phosphorylation/dephosphorylation phenomena are still poorly understood.
In the present study, using western blots analysis, we aimed to profile temporal (0-24 hours) postmortem changes in pig skeletal muscle protein content focusing on their phosphorylation levels; our research target to looking for specific biomarkers that may help the estimation of *post mortem interval* (PMI), as well as evaluate the effects of PMI on specific proteins whose functioning are known to be regulated markedly by phosphorylation. We analyzed many intracellular signaling pathways using antibodies against AKT, ERK, JNK, mTOR, STAT and we found that both total and phosphorylated levels of some proteins decreased with increasing PMI (0-6-12-24 hours). Thus, we plotted the phosphorylation level against time after death, and the non-linear regression was performed in order to find a mathematical model that would provide an accurate estimate of PMI in the early postmortem phase.

P1.24

Electrophysiological characterization of a NEU3 sialidase overexpressing system in H9c2 cell line

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Background: Sialidase NEU3 is a glycohydrolytic enzyme that removes sialic acid residues from gangliosides and it is involved in myotube formation, apoptosis protection and cardiac fibroblasts activation. The H9c2 myoblast cell line, originated from rat embryos ventricular tissue, can be a viable cardiac model for *in vitro* studies. In fact, when cultured in low-serum media with retinoic acid, the small mononucleated myoblasts differentiate into large adult cardiac-like multinucleated cells. In this work, we studied the electrophysiological profile of a H9c2 cell line model stably overexpressing (o.e.) NEU3. **Methods and Results:** H9c2 myoblasts were differentiated as previously described- A neat increase in cell size was observed in NEU3 o.e. myotubes (148.3±24.9 pF, N=8) compared to control myotubes (39.9±3.5 pF, N=12). Patch-clamp experiments in whole-cell configuration were conducted employing different solutions to highlight distinct ionic currents. In these settings, control myotubes exhibited an outward non-inactivating partially TEA-sensitive conductance. Diversely, NEU3 o.e. myotubes showed an inward L-type conductance and an outward current smaller than that seen in control. **Conclusions and Perspectives:** differentiated NEU3 o.e. myotubes showed increased cell size and up-regulated L-type currents. However, it is not clear if NEU3 plays a direct role in the process. Therefore, future experiments will focus on an inducible NEU3 overexpressing system.

P1.25

Altered AQP3 expression and distribution in the epidermis *Hidradenitis suppurativa* patients

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Aquaporin-3 (AQP3) is the most abundant aquaporin channel in the outer epithelial layer of the skin, the epidermis, where it is suggested to act in keratinocyte proliferation, differentiation and migration, skin hydration, would healing, and epidermal water permeability barrier. Accumulating evidence also points to important roles of AQP3 in various skin diseases. Here, we studied the expression and distribution of AQP3 in the epidermis of patients with *Hidradenitis suppurativa* (HS), a chronic recurrent, inflammatory, debilitating skin disease that usually presents after puberty. By immunohistochemistry, skin biopsies from lesion and non-lesion areas of HS patients were compared with healthy skin tissue from subjects undergoing plastic surgery. In healthy skin, epidermal AQP3 was found both in the basal layer, where immunoreactivity was seen both in the plasma membrane and in the intracellular compartment, and in the spinous layer where AQP3 stained almost exclusively over the plasma membrane. No stain was seen in the granular layer. A similar profile was observed in the epidermis of non-lesion areas of HS patients. Considerable alteration of AQP3 expression was seen in the epidermis and hair follicles of HS lesion areas where AQP3 was almost absent in the basal layer by gradually increasing from the bottom to the top of the spinous layer. Our findings suggest involvement of AQP3 in keratinocytes maturation providing novel insights into the understanding of HS pathogenesis.

P1.26

Hearing-loss related genes are deregulated by electromagnetic field exposure in vestibular schwannoma cells

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Hearing loss (HL) is the most common sensory disorder in the world population. One frequent cause of HL is the presence of vestibular schwannoma (VS), a benign tumor of the VIII cranial nerve, arising from Schwann cells (SCs) transformation. The incidence of VSs is particularly high among patients with neurofibromatosis type 2 (NF2), an autosomal-dominant multiple syndrome resulting from mutations in the NF2 gene, coding for the tumor suppressor merlin. However, more than 90% of VSs are sporadic, with unknown pathogenesis. In search of physio-pathologic causes leading to VSs, the attention has been recently focused on the increasing exposure to the environmental challenges, such as the electromagnetic fields (EMFs). Therefore, by means of NGS technology RNA-Seq transcriptomic analysis we investigated the genomic profile and the differential display of HL-related genes of VS cells, after acute and chronic EMF exposure. We found that EMF exposure modified the cell proliferation, in parallel with changes in the intracellular signaling and metabolic pathways related to the translation and the mitochondrial activity. For instance, the expression of some HL-related genes, such as NEFL, TPRN, OTOGL, GJB2 and REST, was deregulated following chronic EMF exposure.
Overall, we studied the molecular mechanisms underlying the physiologic transformation of human SCs into VS. In conclusion, we suggest that the EMF exposure might boost the VS cells onset, then contributing to HL.

P1.27

Simulated microgravity affects cellular physiology: a comparison between adherent and suspension cell lines

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The microgravity alters human physiology with macroscopic effects at organ/systemic level (as muscle and bone mass loss, cardiovascular deconditioning, impairment of immune and reproductive systems) and microscopic effects at cell level (as alteration of cytoskeletal architecture, of metabolic status, of proliferation rate and differentiation capacity). Our study aimed to investigate, on adherent and in suspension cells, the effects induced by simulated microgravity in relation to a different distribution of extracellular forces. The Random Positioning Machine (RPM) was used to simulate microgravity, while TCam-2 male germ cells, H9C2 cardiomyocytes, MC3T3-E1 osteoblasts (all adherent cells), and Jurkat lymphocytes (cells in suspension) were used as cell models. In adherent cells, modifications in cell morphology, decreased proliferation rate, increased anaerobic metabolism, increased intracellular Ca^{2+} and reactive oxygen species (ROS) levels were observed. The use of antioxidants prevented the microgravity-induced effects. The Jurkat cells showed a more homogeneous roundish shape, an increased proliferation rate, a higher metabolic and detoxifying activity resulting in decreased intracellular Ca^{2+} and ROS levels.
These data suggest that extracellular mechanical forces, modified by gravity variations, can induce a phenotypic-specific effect maybe due to a different cell-specific mechano-sensibility and cell-cell or cell-environment interactions.

P1.28

Kir2.1 channel in an oxidative stress-related model of aging neuroglia

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Epilepsy is a chronic disease of the brain and its prevalence increases with age. Metabolic changes, including the production of ROS, may contribute to epilepsy development. Glia plays a crucial role in epilepsy by controlling neuronal hyperexcitability. One of the key roles of glial cells is the spatial buffering of extracellular K^+ ions that are released by excited neurons and transported through glial inwardly rectifying potassium (Kir) channels from extracellular regions of high K^+ to those of low K^+ to inhibit epileptogenesis. Among experimental oxidative stress (OS)-related aging models, exposure to D-galactose (D-gal) is considered the most similar to natural aging. In this study, we investigated the effect of D-gal-induced aging on Kir channel function in glioblastoma U87-MG cells. Screening of all Kir isoforms revealed that the predominant transcript corresponds to Kir2.1 with minor contribution of Kir4.1. D-galactose had no obvious cytotoxicity, but activated OS pathways, namely significantly enhanced lipoperoxidation levels and reduced the abundance of SH groups. Interestingly, D-gal exposure was associated with a decrease of inwardly rectifying K^+ currents sensitive to ML-133, a specific inhibitor of Kir2.1. Our data reveal a novel Kir2.1 channel modulation that is likely to occur in OS. We suggest that inhibition of Kir2.1 in glia cells may alter extracellular K^+ buffering and contribute to OS-related neuronal hyperexcitability and epileptogenesis during aging.

P1.29

Selective inhibition of genomic and non-genomic effects of thyroid hormone tegulates muscle cell differentiation and metabolic behavior

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Thyroid hormones (THs) are key regulators of different biological processes. Their action involves genomic and non-genomic mechanisms, which together mediate the final effects of TH in target tissues. However, the proportion of the two processes and their contribution to the TH mediated effects are still poorly understood. Skeletal muscle is a classical target tissue for TH, which regulates muscle strength and contraction, as well as energetic metabolism of myofibers. Here we address the different contribution of genomic and non-genomic action of TH in skeletal muscle cells by specifically silencing the deiodinase Dio2 or the b3-Integrin expression via CRISPR/Cas9 technology. We found that myoblast proliferation is inversely regulated by integrin signal and the D2-dependent TH activation. Similarly, inhibition of the nuclear receptor action reduced myoblast proliferation, confirming that genomic action of TH attenuates proliferative rates. Contrarily, genomic and non-genomic signals promote muscle differentiation and the regulation of the redox state. Taken together, our data reveal that integration of genomic and non-genomic signal pathways finely regulates skeletal muscle physiology. These findings not only contribute to the understanding of the mechanisms involved in TH modulation of muscle physiology but also add insight into the interplay between different mechanisms of action of TH in muscle cells.

P1.30

The response of digestive cells of *Mytilus galloprovincialis* after exposure to thiacloprid

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Nowadays, the correct and improper use of pesticides is on the rise. Thiacloprid is a neonicotinoid used in commercial pesticides that affects nicotinic acetylcholine receptors, which are highly presented in insects, as well as in other invertebrates. It is widely used in intensive agriculture and the risk of finding it in wastewater is very high.
The purpose of this work was to evaluate the possible risks posed by thiacloprid on *Mytilus galloprovincialis* by exposing mussels acutely (7 days) and chronically (20 days) to different concentrations of pollutants .
The following physiological parameters were monitored:
-Cell viability, through neutral red assay in cells of the digestive gland;
-Regulation of volume decrease (RVD) in the cells of the digestive gland;
-Antioxidant parameters, such as superoxide dismutase (SOD) and catalase (CAT) in mussel digestive gland.
Following acute exposure to the pollutant, the results did not show cell damage, while after chronic exposure the activity of antioxidant enzymes were altered.
Our data suggest that metabolism was impaired after chronic exposure to very low concentrations of thiacloprid, but *M. galloprovincialis* resists showing no other physiological alterations.
This could be a risk, as mussels could accumulate thiacloprid in the tissues and be ingested indirectly by humans, because these organisms are part of the food chain.

P1.31

Another brick in the wall of cardioprotection: the role of Catestatin via macrophages immunosuppression

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Inflammation and excessive catecholamine production are crucial milestones in the development of hypertension. Hypertensive patients show reduced plasma levels of Catestatin (CST), a bioactive peptide derived from the cleavage of Chromogranin A (CgA), and, in a mouse model, hypertension symptoms are reduced by administration of CST. The present study aimed to deepen the role of CST in regulating cardiovascular function by using mice with knockout of the region of the CgA gene coding for CST (CST-KO). CST-KO mice developed hypertension and displayed left ventricular hypertrophy, marked macrophage infiltration of the heart and adrenal gland, elevated levels of proinflammatory cytokines and catecholamines. Cardioprotection induced by ischemic preconditioning was abolished in CST-KO mice and intraperitoneal injection with CST was able to reverse these phenotypes. Experiments with chlodronate depletion of macrophages and bone marrow transfer were performed. Without CST macrophages were more reactive, infiltrated the heart, and altered the ultrastructure, and the physiological/molecular makeup of the myocardium. Macrophages and chromaffin cells were able to produce CST that reduced inflammation, underlining the antihypertensive effects of CST. CST emerges as a key mediator of the cross talk between systemic and cardiac inflammation in hypertension, which hence plays a central role in cardiovascular homeostasis by regulating the immunoendocrine axis.

P1.32

Protective effect of hydroxytyrosol on toxicity induced by the environmental pollutant dichlorodiphenyldichloroethylene (DDE) in liver cells in vitro (HepG2)

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Dichlorodiphenyldichloroethylene (DDE) is the most persistent metabolite of DDT with endocrine disrupting activity due to the bioaccumulation in fatty tissues of organisms. DDE induces lipotoxicity by increasing reactive oxygen species with activation of apoptotic processes. Hydroxytyrosol (Hty), polyphenol of extra virgin olive oil, is able to act on cellular metabolism by mitigating the damage induced by environmental pollutants.
The aim of this study was to evaluate Hty effect on the viability reduction induced by DDE on cultured liver cells (HepG2). Cells were stimulated with DDE doses of 30mM and 100mM, or simultaneously with the following DDE and Hty doses: DDE 30mM + Hty 50mM, DDE 30mM + Hty 100mM, DDE 100mM + Hty 50mM, DDE 100mM + Hty 100mM. Cell viability and Caspase-3 content were analysed by MTT and western blot analysis, respectively. The results suggested that DDE induced a dose-dependent decrease in cell viability vs control, whereas Hty coincubation induced a dose-dependent increase vs DDE. Cell co-treated with 30mM DDE and 100mM Hty showed a values of cell viability similar to control cells. Moreover, analysis of caspase 3 content showed that coincubation with Hty reduced the expression of caspase 3 compared to cells treated only with DDE. In conclusion, the preliminary data presented in this study suggested that Hty could have a protective effect against cellular damage induced by environmental pollutants.

P1.33

Functional characterization of transport across peritoneal mesothelium

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While the physiological role of the peritoneum is well known, in clinical settings, it can be exploited as a semipermeable membrane in peritoneal dialysis (PD). It has been hypothesized that the endothelium of peritoneal capillaries is the main limiting barrier to water flux occurring during PD. However, the presence of the water channel AQP1 at the plasma membrane of mesothelial cells suggests that mesothelium may represent a second selective barrier to water diffusion in PD. To demonstrate that, we characterized a cell line of human mesothelium (HMC), by immunofluorescence and by Ussing chambers measurements, and investigate the transepithelial water transport, in the presence or in the absence of AQP1, by an electrophysiological approach based on measurements of TEA^+ dilution in the apical bathing solution, by means of TEA^+-sensitive microelectrodes. TEER measurements, the presence of tight junctions and a transepithelial vectorial Na^+ transport, indicated the establishment of a polarized monolayer. Real-time measurements of transmesothelial water flux, in response to an increase of osmolarity in the apical solution, indicated that, in the presence of AQP1, the rate of TEA^+ dilution was up to four-fold higher than in the absence. These data suggest that the mesothelium could represent a barrier controlling water transport in PD through AQP1. This implies that PD solutions or pharmacological treatments aimed at preserving AQP1 levels, can maintain high efficiency of PD.

P1.34

Rescue of the homeostasis in cells expressing the pathogenic LMNA variant R321X by nonsense mutation readthrough and the ER stress inhibition

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Nonsense *LMNA* gene mutations can be associated to severe forms of inherited cardiomyopathy, but the molecular mechanisms involved in this disease and the possible therapeutic approaches have not been systematically studied yet. We have previously shown that a truncated mutant of Lamin A, R321X, identified in family members affected by severe a dilated cardiomyopathy, mislocalized in the endoplasmic reticulum (ER) inducing ER stress and apoptosis, when expressed in cell lines. Here, we tried to rescue cellular functions in R321X-expressing cells with different approaches. We found that Gentamycin was able to read through the premature stop codon in *lmna* gene responsible for the expression of R321X LMNA variant inducing the synthesis and the correct localization of the full-length LMNA protein in R321X-expressing cells in a dose-dependent and sustained manner. Moreover, we found that Salubrinal, a known ER stress inhibitor, significantly downregulated the expression levels of the ER stress markers CHOP and p-PERK in R321X-expressing cells. The cleavage of the caspase-3 substrate, PARP, was also significantly inhibited in R321X-expressing cells treated with Salubrinal. Finally, either Gentamycin or Salubrinal inhibited cell apoptosis in R321X-expressing cells. In conclusion, here we provided the proof of concept for the efficacy of different therapeutic approaches for patients carrying R321X pathogenic LMNA variant.

P1.35

In vivo treatment with calcilytic reverses the reduced expression of AQP2 and the higher AQP2-targeting miRNA-137 levels in Calcium-Sensing Receptor (CaSR) Knock-In mice mimicking Autosomal Dominant Hypocalcemia (ADH)

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High concentrations of urinary calcium counteract vasopressin action *via* the activation of the Calcium-Sensing receptor (CaSR) expressed in the luminal membrane of collecting duct cells, which impairs the trafficking of aquaporin-2 (AQP2). In line with these findings, here we provide evidence that, with respect to WT mice, CaSR Knock-in mice (KI) mimicking Autosomal Dominant Hypocalcemia (ADH), display a significant decrease in total content of AQP2 associated with significantly higher levels of AQP2 phosphorylation at Ser261, a phosphorylation site involved in AQP2 degradation. Interestingly, KI mice also had significantly higher levels of phosphorylated p38-MAPK, a downstream effector of CaSR and known to phosphorylate AQP2 at Ser261. Moreover, ATF1 phosphorylated at Ser63, a transcription factor downstream of p38MAPK, was significantly higher in KI. In addition, KI mice had significantly higher levels of AQP2-targeting miRNA-137 consistent with a posttranscriptional downregulation of AQP2. *In vivo* treatment of KI mice with the calcylitic JTT-305, a CaSR antagonist, increased AQP2 expression and reduced AQP2-targeting miRNA-137 levels in KI mice. Together, these results provide a direct evidence for a critical role of CaSR in impairing both short-term vasopressin response by increasing AQP2-pS261, and AQP2 abundance, via p38MAPK-ATF1-miR137 pathway.

P1.36

Specific activation of BK potassium channels has no effect on cell viability, proliferation and migration in melanoma and pancreatic adenocarcinoma cell lines

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Potassium channels have emerged as regulators of carcinogenesis, thus introducing possible new therapeutic strategies in the fight against cancer. In particular, the large-conductance Ca^{2+}-activated K^{+} channel, often referred to as BK channel, is involved in several tumor-associated processes. Since it is assumed that BK channel activation leads to cell hyperpolarization, as well as a modulation of cancer progression, we investigated the effect of BK channel activation in IGR39 (melanoma) and Panc-1 (pancreatic tumor) cell lines, employing three different activators, namely NS-11021, NS-19504 and BMS-19011. NS-11021 potently induced the activation of BK channels. On the contrary BK channel activation by NS-19504 and BMS-19011 was less effective. Moreover, none of these BK activators showed potential anti-tumor effects in tumor-associated processes, such as cell proliferation, survival and migration. Surprisingly, we found that NS-11021 and BMS-19011 led to an increase of intracellular Ca^{2+} concentration, complicating any interpretation of their role as specific BK channel activators. Only NS-19504 activated BK channels without an increase of intracellular Ca^{2+} levels in both cell lines. Thus, we conclude that the specific activation of BK channels in these tumor cell lines has no beneficial anti-tumor effects. Importantly, our results raise an alarm flag regarding the use of potential specific BK channel openers as anti-tumor agents.

P1.37

Interaction of the novel endogenous peptide Cateslytin with Toll Like Receptor 4: mechanism of action and cardioprotection

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Toll-like receptor 4 (TLR4) is a key member of TLR family and activates innate and adaptive immune cells. Expressed on the surface of host cardiomyocyte, TLR4 is crucially activated during myocardial inflammation and other physiopathological conditions, such as lipotoxicity. Among the endogenous Chromogranin A-derived peptides, Cateslytin (Ctl) acts as regulator of the innate immunity and host defence peptide, being effective against many microbial strains. Here, we investigated the physiological properties of Ctl in H9c2 cardiomyocytes exposed to LPS infection. We showed that, following LPS, Ctl increased cell viability and mitigated cytotoxicity, inflammation and oxidative stress by targeting TLR4/ERK/JNK/p38-MAPK pathway, regulating NFkB p65/p52 and COX2 expression and repressing IL-1β, IL-6, TNF-α and NOS2 mRNA. Molecular docking simulations strongly suggested that Ctl modulated TLR4 through a direct binding to MD-2. Recent evidences indicated that as LPS, palmitate (PA), the most abundant circulating saturated fatty acid, direct binds to MD-2 to induce myocardial and systemic inflammation and insulin resistance. Therefore, our ongoing studies are testing the ability of Ctl to mitigate PA-induced lipotoxicity in cardiomyocytes by competing with PA for the binding site of MD-2 during dysmetabolism-induced inflammatory response. Our evidences suggest that Ctl may influence the biological activity of TLR4 acting as protective agent able to switch-off inflammation.

P1.38

The β-catenin/c-Myc axis acts as a molecular switch in the regulation of the autophagy process during hyperammonemia in the Huh7 cell line

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Autophagy is a cellular catabolic process aids in maintaining cellular homeostasis and supplying substrates for energy generation. Ammonia is a diffusible compound that is produced from the catabolism of nitrogen-containing molecules and is efficiently converted in non-toxic urea and glutamine by the healthy liver. Ammonia has a dual role in autophagy, it acts as an inducer at low concentrations and as an inhibitor at high concentrations. At present, little is known about the cellular mechanisms responsible for this functional switch. Wnt/β-catenin signaling is emerging as a forerunner for its roles in many aspects of liver biology. This prompted us to investigate the functional association between the β-catenin/c-Myc axis and the modulation of autophagy by hyperammonemia, in hepatocarcinoma Huh7 cells.
Specifically, we found that *in vitro*, at high concentrations of ammonia, the autophagy process is inhibited and the expression of β-catenin and c-Myc are both reduced, while on the contrary, the opposite effect is observed at low concentrations of ammonia when the autophagy process is active. Furthermore, hyperammonemia, by damaging autophagic flux, induces also lipid accumulation into lipid droplets, due to reduced lysosomal lipolysis. In Huh-7 cells, knockdown of c-Myc induced autophagy inhibition and lipid accumulation. Overall, these results demonstrate the functional role of the β-catenin/c-Myc axis in mediating the effect of hyperammonemia on autophagy.

P1.39

Effect of Quercetin on d-Galactose aging model in human erythrocytes

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Blood is not only a supplier of O_2 and nutrients for tissues, but also removes metabolic waste and oxidative species, thus playing a special role in aging. Aging may exert its impact on plasma membrane and, among integral membrane proteins, on the Cl^-/HCO_3^- exchanger, which is involved in gas exchange and acts as a major site of cytoskeleton attachment to the membrane. The aim of the present work was to verify the possible beneficial effect of 10 µM Quercetin (Q), a polyphenolic flavonoid compound with strong antioxidant activity, on a model of accelerated aging represented by human erythrocytes exposed to 50 or 100 mM d-Galactose for 24 hours. The rate constant for SO_4^{2-} uptake through the anion exchanger Band 3 protein (B3p) has been monitored along with lipid peroxidation, SH- groups oxidation and glycated hemoglobin (A1c) levels, all of which are known to critically affect B3p function. Our results show that: i)d-Gal accelerates the rate constant for SO_4^{2-} uptake, induces lipid peroxidation, SH-group oxidation and A1c; ii)Q attenuates d-Gal effect by preventing the rate constant acceleration, lipid peroxidation and total SH- group oxidation, rather than A1c formation.
These findings may help to clarify the mechanism of aging in human erythrocytes and propose Q as a potential dietary supplement for novel therapeutic strategies to counteract oxidative age-related disturbances linked to B3p dysfunction. Future experiments are needed to elucidate Q possible antiglycant effect.

P1.40

Sphingosine-1-phosphate is involved in lung cancer-related inflammation

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Sphingosine-1-phosphate (S1P) is a membrane-derived bioactive phospholipid exerting a multitude of effects on respiratory cell physiology and pathology through five S1P receptors (S1PR1-5), although its exact mechanism is still elusive and contradictory.
In our previous studies, we found that S1P induced proliferation of epithelial lung cancer cells through a S1PR3/SPHK II-dependent intracellular/intranuclear signalling. In this study we aimed to shed light on the role of S1P, whose levels are elevated in lung cancer patients' plasma, as a modulator of tumor microenvironment, focusing on its effect on circulating cells.
We used peripheral blood mononuclear cells (PBMCs) isolated by healthy volunteers and lung cancer patients, to compare physiological vs pathological conditions. Cells were stimulated with S1P 10nM and the release of pro-inflammatory cytokines was evaluated.
We found that lung cancer-derived PBMCs expressed higher levels of S1PR3 and active ceramidase, compared to healthy cells. Moreover, the administration of S1P induced the release of TNFα, IL-6 and IL-8 from lung cancer- but not from healthy-derived PBMCs in a ceramidase and SPHKs-dependent manner. Interesting, only IL-6 release was S1PR3 dependent.
Our data suggest that S1P regulate the pro-inflammatory signalling in pathological conditions, but not in physiological conditions where S1PR3 is poor expressed and ceramidase enzyme is inactive, implying a protective role of S1P in physiological conditions.

P1.41

Genetic ablation of G protein-gated inwardly rectifying K^+ channels improves heart rate in Angiotensin II-induced sinus bradycardia mice model

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Background: Secondary sinus bradycardia is associated with heart failure (HF) and is responsible for sudden death in hospital. We recently reported that genetic ablation of G protein gated K^+ (I_{KACh}) channels ($Girk4^{-/-}$) prevents sinus bradycardia induced by intensive exercise training in mice.
Objective: To test if genetic ablation of I_{KACh} prevents bradycardia in Angiotensin II (AngII)-induced HF mouse model.
Methods: Control wild-type (WT) and $Girk4^{-/-}$ mice were assigned to NaCl- or AngII- treated groups. In vivo ECG recordings and patch clamp experiments in isolated SAN cells were performed, respectively, at 5 and 8 weeks after the beginning of treatments.
Results: AngII-treated WT mice presented a significantly lower heart rate in comparison to the other groups. The "funny" current (I_f) density was significantly decreased (37% at -135mV) only in SAN cells derived from AngII-treated WT mice. L-type Ca^{2+} peak current (I_{CaL}) was reduced by 37% in AngII-treated WT group and by 17% in AngII-treated $Girk4^{-/-}$ group compared to NaCl-treated counterparties. Similarly, T-type Ca^{2+} peak current (I_{CaT}) was diminished by 38% in AngII-treated WT group and by 14% in AngII-treated $Girk4^{-/-}$ group compared to NaCl-treated counterparties.
Conclusion: Genetic ablation of cardiac I_{KACh} prevents AngII-induced sinus bradycardia by restoring I_f and partially rescuing I_{CaL} and I_{CaT}.

P1.42

Oxidative damage and mitochondrial functionality in hearts from KO-UCP3 mice housed at thermoneutrality

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The primary function of mitochondria is ATP synthesis, but they are also involved in critical processes to cell function and survival as protection from oxidative stress. Uncoupling proteins (UCPs) are homologous proteins belonging to a subfamily of mitochondrial anion carriers. The first UCP (UCP1, located in brown adipose tissue) was identified in 1976, and later other UCPs have been discovered, among which UCP3, which is present in metabolically very active tissues such as skeletal and cardiac muscle. Among the many functions attributed to this molecule, the potential to protect against oxidative stress is of particular importance, especially for organs as the heart. We evaluated the effects of UCP3 ablation on heart metabolic capacity and oxidative stress using UCP3 KO and WT mice. In most researches, the housing temperature of the animals is below thermoneutrality. Therefore, they are exposed to cold that affects both ROS production and oxidative stress. So, to avoid overlapping results, we kept the animals at thermoneutrality. Under these conditions, we observed in KO mice an increase in the cardiac level of mitochondrial oxidative stress associated with higher levels of lipid and protein oxidative stress markers than in WT. Furthermore, reduced mitochondrial respiratory capacity was observed in KO mice. These results show that UCP3 contributes to mitigating oxidative stress and maintaining mitochondrial function.
KEYWORDS: UCP3, oxidative stress, mitochondria

P1.43

Disruption of the ser-269 in the AQP2 abolishes the gain-of-function phenotype in renal cells expressing the V2R-R137L/C mutants causing Nephrogenic Syndrome of Inappropriate Antidiuresis (NSIAD): functional implication in AQP2 trafficking

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NSIAD is a rare X-linked condition associated with gain-of-function mutations of the V2 vasopressin receptor (V2R). It causes hyponatremia, decreased serum osmolality, and inappropriately increased urinary osmolality. We have recently demonstrated that the V2R mutant R137L causing NSIAD, signals through an alternative pathway that increases AQP2 membrane targeting independently of S256 phosphorylation. To better investigate this alternative PKA-independent pathway, Flp-In T-REx Madin-Darby canine kidney (FTM) cells, stably transfected with V2R mutants (R137L, R137C) and AQP2-wt or non-phosphorylatable AQP2-S269A/AQP2-S256A, were used as cellular models. All activating V2R mutations presented constitutive apical membrane expression of AQP2-wt and significantly higher basal water permeability. In addition, V2R-R137L/C showed significantly higher activity of ROCK, a serine/threonine kinase previously suggested to be involved in S269-AQP2 phosphorylation in these mutants. Interestingly, FTM cells expressing V2R-R137L/C mutants and the non-phosphorylatable AQP2-S269A lost the gain-of-function phenotype which was instead retained when AQP2-S256A was co-expressed indicating that AQP2 trafficking was independent of S256 phosphorylation.
These data indicate that the constitutive AQP2 trafficking associated with the gain of function V2R-R137L/C mutants causing NSIAD is PKA-independent and requires an intact ser-269 in the AQP2 protein under the control of ROCK phosphorylation.

P2.1

Indicaxanthin from *Opuntia Ficus Indica* fruit prevents the glucose dismetabolism in obese mice

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In recent years there has been a growing interest in natural compounds contained in food, able to counteract metabolic dysfunctions (MD), including insulin resistance (IR). Indicaxanthin (Ind), a betalain from *Opuntia ficus indica* fruit, exerts anti-oxidative and anti-inflammatory actions both *in vivo* and *in vitro*. Because inflammation, oxidative stress and IR are strictly related, the aim of this study was to explore the effects of Ind, purified as detailed in the Italian Patent Application No.102021000015167 filed on 10.06.2021, in an *in vivo* model of MD related to IR. Mice were so grouped: 1. Mice fed a standard diet (STD) for 14 weeks; 2. Mice fed a high fat diet (HFD) for 14 weeks; 3. Mice fed a HFD for 10 weeks and then receiving Ind for 4 weeks. Body weight (BW), food intake (FI), fat mass (FM), glucose dysmetabolism, inflammatory and oxidative status in liver and visceral adipose tissue (VAT) were analyzed. Ind significantly reduced BW, FI, FM and VAT hypertrophy. It also improved significantly fasting glycaemia, insulinaemia, glucose tolerance and exogenous insulin sensitivity. Ind decreased the reactive oxygen and nitrogen species, malondialdehyde and NO levels in both VAT and liver and it reduced TNF-α, CCl-2, F4-80 gene expression, p65, p-JNK, COX-2, i-NOS protein levels and VAT crown-like structures and hepatic inflammatory foci. The present results indicate that Ind is able to counteract IR in obese mice *via* anti-oxidative and anti-inflammatory mechanisms.

P2.2

Adipose and hepatic aquaglyceroporins in energy balance in health and disease

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Glycerol is an important intermediate in energy metabolism, being a key substrate for gluconeogenesis and direct source of glycerol-3-phosphate for triglycerides synthesis. Aquaglyceroporins (AQP3, 7, 9 and 10) constitute a group of aquaporin membrane channels that facilitate the movement of glycerol and other solutes in addition to water into or out of cells. Fat is a major source of glycerol released by adipocytes through AQP7 and 3. Lipolytic glycerol flows to the liver where it is imported by hepatocytes through AQP9. The functional significance of AQP10 in fat tissue and liver is unclear. Although with distinctions between rodents and human, adipocyte and hepatocyte aquaglyceroporins are controlled by insulin and leptin via the PI3K/Akt/mTOR signaling cascade. Estrogens exert negative regulation on fat and hepatic aquaglyceroporins explaining their sexual dimorphism in energy homeostasis. Adipose and liver AQP7 and AQP9 play roles in energy balance disorders. AQP7 deficiency leads to abnormal triglycerides accumulation in fat tissue and adult onset of obesity while alterations in hepatic AQP9 level are seen in animal models and patients with diabetes, obesity and/or fatty liver disease. Hepatocyte AQP9 is involved in the lipid-lowering activity exerted by the nutraceutical silybin through modulation of autophagy. Potent and isoform-specific inhibitors of AQP3, 7 and 9 are available with potential pharmacological development in the treatment of energy balance disorders.

P2.3

Long-term effect of a daily consumption of *Moringa oleifera* leaf powder on blood pressure: a 3-months pilot study in Saharawi diabetic women

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Hypertension in diabetes is a risk factor for micro- and macrovascular complications. Among the Saharawi population, diabetes and hypertension management is poor. *Moringa oleifera* leaves (MO) have been suggested to have a hypotensive effect. We performed a 3-months study in Saharawi diabetic women, whose secondary aim was to evaluate the MO effect on blood pressure. Sixty-two patients were randomly assigned to experimental (EXP), receiving 10g of MO to consume in the main meals, or control group (CNT). A clinical, anthropometric, laboratory and blood pressure assessment was performed at baseline and after 3 months. MO effect on blood pressure was tested using linear mixed models employing treatment, time and a treatment#time interaction as fixed-effect predictors and the patient as random effect. Fifty-three women (85.5%, age 62±16y, BMI 28.8±5.9kg/m^2) completed the study. 87% had HbA1c>7% and 66% were hypertensive. Baseline systolic and diastolic blood pressures did not differ between groups. We observed no significant effect of treatment#time interaction, and at the end of the study blood pressure did not differ between groups. However, testing the simple effect of time at each treatment group, we found that systolic blood pressure significantly decreased in EXP (-5.6 mmHg, 95%CI: -10.7,-0.4), but not in CNT (-3.5 mmHg, 95%CI: -10.6,3.6). In conclusion, MO in the long-term may improve blood pressure in diabetics at high risk for complications. Clinical trials are required.

P2.4

Differences in fat oxidation rates during moderate aerobic exercise among healthy aged and younger women and mildly disabled women with multiple sclerosis: a pilot study

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In women, fat oxidation rates during moderate aerobic exercise decline with age, mainly due to decay in estrogen levels. Reduced mitochondrial function is also accounted for impaired lipolysis leading to the early-onset fatigue, as demonstrated in the elderly and in multiple sclerosis (MS). Aim of this study was to test differences in substrate utilization during moderate aerobic exercise between healthy postmenopausal and younger women, and to compare them to a group of mildly disabled younger women with MS, taken as a pathophysiological model of mitochondrial dysfunction leading to impaired lipolysis.
An open-circuit gas-analyzer was employed during (1) an incremental cycling exercise test to determine the first ventilatory threshold (VT1), and (2) a 45-minute constant-load exercise at VT1 to quantify substrate oxidation.
Postmenopausal women (n=6; aged 61.8±5.4) showed significantly lower fat oxidation rates than younger women (n=6; aged 43.8±11.3) by 42.4% (p=0.009), while proving similar (-5.4%; p=0.99) to younger women with MS (n=6; aged 41.2±11.5). By contrast, no differences in carbohydrate and protein oxidation among groups emerged.
These preliminary results suggest that in postmenopausal women, along with estrogen decline, impaired mitochondrial function may contribute to reduced lipolysis and early onset of fatigue. If confirmed over a larger sample, these findings may be relevant to future research investigating the pathophysiology of fatigue in the elderly.

P2.5

Persistence of fructose-induced gut dysfunction after switching to a healthy diet

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The link between increased fructose intake and induction of gut and liver dysfunction has been established, while it remains to be understood whether this damage is reversible, particularly in the young population. To this end, young (30 days old) rats were fed a fructose-rich or control diet for 3 weeks, to highlight the early response of gut and liver to increased fructose intake. After this period, fructose-fed rats were returned to control diet for 3 weeks and compared to rats that received the control diet for the entire period, to identify whether fructose-induced changes in the gut-liver axis persist or not. GLUT- 5 and occludin were assessed in ileum and colon. Markers of inflammation and redox homeostasis, as well as fructose and uric acid levels were also evaluated in ileum, colon and liver. From the whole data, it emerges that metabolic derangement elicited by fructose-rich diet, even after a brief period of intake, is fully reversed in the liver by a period of fructose withdrawn, while the alterations persist in the gut, especially in the ileum. In conclusion, given the increasing consumption of fructose-rich foods in young populations, the present results highlight the risk arising from gut persistent alterations even after the end of a fructose-rich diet. Therefore, dietary recommendations of reducing the intake of this simple sugar is mandatory to avoid not only the related metabolic alterations but also the persistence of these detrimental changes.

P2.6

A machine learning approach to evaluate taste sensitivity

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Taste sensitivity greatly varies in humans. Psychophysical and electrophysiological methods are used to evaluate inter-individual variability. We present a machine learning (ML) approach for the automatic identification of taste sensitivity to the prototypical stimulus, 6-n-propylthiouracil (PROP) by 84 subjects. Supervised Learning (SL) classifier was used to automatically classify the subjects as belonging to PROP taster categories. The following features were included in the system: psychophysical ratings, taste sensitivity for taste qualities, papilla density, genotypes of genes encoding specific taste receptors, age, gender, BMI and smoking status. The automatic classification of PROP taster status was obtained with a high precision (97%). A strong correlation of PROP taster status with the taste ratings, gene genotypes and papilla density was found. The features were classified in order of importance as follows: PROP paper disk (50 mM), PROP solutions (0.32 mM and 3.2 mM), PAV/PAV genotype (mostly to classify super-tasters), AVI/AVI genotype (mostly to classify non-tasters), papilla density (a high value pushes toward super-taster prediction). These results, by showing that the SL approach allows to obtain an automatic and high precision classification of PROP taster status of subjects, suggest that it may represent an objective and reliable tool for taste physiology studies, with applications ranging from basic science to medicine.

P2.7

The vitamin-E derived delta-tocotrienol overcomes drug-resistance by triggering necroptosis in prostate cancer cells

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Vitamin E–derived tocotrienols (TTs) exert anticancer activity in different human cancer cells. Our previous studies have demonstrated that δ-TT induces anti-proliferative/pro-apoptotic effects in castration-resistant prostate cancer (CRPC) cells, DU145 and PC3. Currently chemotherapeutic agents mainly inhibit tumor growth by induction of apoptosis. However, defects in apoptotic signaling in cancer cells frequently lead to drug resistance, which has already been the main cause of chemotherapy failure. Necroptosis, a non-apoptotic form of regulated cell death, is considered as a new approach to overcome chemotherapeutics resistance. This study evaluated the involvement of necroptosis in anticancer activity of δ-TT in three human prostate cancer cell lines (PC3, DU145 and docetaxel-resistant DU145 (dr-DU145). Our results demonstrated that δ-TT induced significant necroptosis by activating receptor-interacting serine/threonine-protein kinase 1(RIPK1) and subsequently promoting the translation of mixed-lineage kinase domain-like protein (MLKL) from cytoplasm to plasma membrane in our cancer cell models. δ-TT-induced necroptosis was inhibited by a RIPK1 inhibitor necrostatin-1, further supporting a role of RIPK1 in the effects of δ-TT.
In summary, these data show that δ-TT induces necroptosis not only in PC3 and DU145 cells but also in dr-DU145 cells, indicating that δ-TT could be considered a potential anticancer compound in drug-resistant prostate cancer cells.

P2.8

Aluminum chronic exposure affects the redox state of zebrafish gills

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Aluminum (Al) is considered a dangerous pollutant especially for aquatic organisms since the phenomenon of acid rain makes the metal more available in water bodies. Several studies have shown that Al can produce toxic effects in fish by altering physiological and biochemical processes. The toxicity mechanisms are poorly understood and it has been reported that the metal can potentiate the pro-oxidant effects of Fe and Cu, which are present in most cell compartments. As a consequence, reactive oxygen species (ROS) levels increase so altering tissue redox state. Gills are the first tissue to get into contact with environmental Al but, to date, very poor information is available about the Al effects on their oxidative state. Here we report the effects of chronic exposure to 11 mg/L of Al for 10, 15, and 20 days in gills of zebrafish adults. This experimental model is a bioindicator for the evaluation of environmental pollutants' ecotoxicological effects. Specifically, ROS content, antioxidant enzymes activity, oxidative damage to lipids and, *in vitro* susceptibility to oxidative stress were evaluated. The results indicate that Al increases ROS levels, lipid hydroperoxides, and the activity of antioxidant enzymes such as glutathione peroxidase and glutathione reductase. However, the antioxidant system is more efficient after 20 days of exposure suggesting an adaptive mechanism that makes the organisms less susceptible to oxidative stress induced by Al exposure.

P2.9

Lumbricus terrestris as a simplified animal model to approach the study of the intrinsic contractility of vertebrate lymphatic vessels: a pilot study

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Many *anellidae* species possess a closed blood circulation endowed with contracting vessels and/or pseudohearts. *L. terrestris* has a contracting dorsal vessel tributing to a series of five doublets of pseudohearts located in the 7 to 11 body segments region. The origin of their contractility is supposed to be myogenic and their ensemble contracting activity seems loosely arranged in a coordinated manner.
Albeit being a closed circulatory system, the contracting features of its rostral portion closely resemble the spontaneous contracting behaviour of diaphragmatic lymphatics loops that are being intensively studied in our lab. Therefore, we started a pilot study on the feasibility of the use of *L. terrestris* as a candidate model for the study of the finest details of the intrinsic contractility of diaphragmatic lymphatic loops.
In this pilot study we found that contraction frequency at 24°C (17.7 ± 1.1 bpm), susceptibility to temperature changes and pseudo-coordinated contraction waves closely resemble those already documented diaphragmatic lymphatic loops. Moreover, earthworm vessel contractions are stable for prolonged time (7% variation in 30 minutes); vessel diameter is of comparable size (428.1 ± 25.2 µm) to the larger lymphatic vessels and they are easily accessible for intraluminal pressure measurements. Further studies are currently aimed at investigating the pharmacological profile of the pacemaker mechanism to further confirm the working hypothesis

P2.10

Non-invasive assessment of intrinsic positive end-expiratory pressure

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Assessment of intrinsic positive end-expiratory pressure (PEEPi) in spontaneously breathing subjects requires an esophageal balloon (ES) to estimate the end-expiratory sudden drop of alveolar pressure (P_{alv}) corresponding to PEEPi. With the advent of electronic compensation, P_{alv} can be estimated also plethysmographically, opening the possibility to assess PEEPi non-invasively. The aim of this study is to develop a procedure to identify PEEPi on plethysmographic P_{alv} tracings and to compare the result of its application with ES-derived data ($PEEPi_{es}$) from the literature.
60 COPD patients underwent plethysmography before and after bronchodilation (BD). 35 patients exhibited tidal expiratory flow-limitation (FL), a condition likely to induce PEEPi. Plethysmographic PEEPi ($PEEPi_{pl}$) was identified as the sudden drop of P_{alv} immediately before end-expiration by an automated algorithm.
Before BD, 34 COPD patients with FL and 11 without FL presented $PEEPi_{pl}$. In patients with $PEEPi_{pl}$, $PEEPi_{pl}$ was greater in the presence than in the absence of FL (3.8 (1.8) versus 2.0 (1.4) cmH_2O, P=0.007). On average, after BD $PEEPi_{pl}$ decreased by ~30%. $PEEPi_{pl}$ was inversely correlated with IC%p and FEV_1%p, and positively correlated with dyspnea at rest ($p<0.001$). $PEEPi_{pl}$ values were similar to $PEEPi_{es}$ previously measured (Haluszka 1990; Dal Vecchio 1990).
In conclusion, although a direct $PEEPi_{pl}$-$PEEPi_{es}$ comparison is needed to validate the technique, non invasive assessment of PEEPi seems feasible.

P2.11

Investigation on accumulated PFAS impact on the stress response in freshwater fish of the Veneto Region

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Understanding the uptake of non-native environmental substances is essential to evaluate their potential risk for aquatic ecosystems. Perfluoroalkylated substances (PFA) are incredibly persistent in the environment due to the perfluorinated tail and their anionic head group. Due to the movement in the water, fish are highly affected by pollutants, where the intake focuses on contaminated food sources and dermal absorption. The stress response of a sessile and a potamodromous fish species were analyzed to evaluate the health impact on riverine fish. Padanian goby (*Padogobius bonelli*) and chub (*Squalius cephalus*) were caught in three different rivers with a high, medium and low concentration of PFA in the Veneto region close to Vicenza. The stress response was studied through the HPI axis (hypothalamic-pituitary-internal axis). The acute stress response was determined by the cortisol blood levels, whereas the chronic stress cortisol level was appraised in the scales, both measured by RIA. Furthermore, the correspondence between cortisol blood level and changes in the protein gene expression in the target organs was investigated. Oxidative stress was evaluated by apoptosis and DNA breaks and the gene expression of the protein components of the antioxidant systems by using qRT-PCR. Interaction of immune cells in the tissue of the intestine was observed via immune histology.

P2.12

3,5-diiodo-L-thyronine (T2) counteracts inflammatory state in rats receiving long-lasting high-fat diet through activation of Irisin-SIRT1 pathway

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Over the last decades, 3,5-diiodo-thyronine (T2) has received marked attention as it was demonstrated to be a biologically active compound. Several studies indicated that T2 exhibits important biological effects in different tissues such as liver, skeletal muscle, heart and adipose tissues. Specifically, when exogenously administered, T2 increases the resting metabolic rate and elicits beneficial hypolipidemic effects in high fat diet fed rats. In the present study, we focused our attention on T2 actions aimed at improving the adverse effects of long-lasting HFD such as the inflammatory response. For this purpose, three groups of rats were used throughout: i) receiving a standard diet for 14 weeks; ii) receiving a HFD for 14 weeks, and iii) receiving a HFD for 14 weeks with a simultaneous daily injection of T2 for the last 4 weeks. The results showed that T2 administration ameliorated the expression profiles of pro- and anti-inflammatory cytokines, reduced macrophage infiltration in visceral white adipose tissue (vWAT), reduced lymphocytes recruitment and affected hypoxia and angiogenesis. Furthermore, T2 administration increased serum levels of Irisin and protein levels of SIRT1 in vWAT. This study demonstrates that T2 is able to counteract some adverse inflammatory effects caused by a long-lasting HFD by activating Irisin-SIRT1 signaling pathway.

P2.13

Olfactory sensitivity is associated with body mass index and polymorphism in the voltage-gated potassium channels Kv1.3

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Smell strongly contributes to food choice and its hedonistic evaluation. A reduction or loss of smell has been related to malnutrition problems, resulting in excessive weight loss or gain. Voltage-gated potassium channels Kv1.3 are widely expressed in the olfactory bulb and contribute widely to the value of the resting membrane potential and to the frequency of action potentials. Mutations in the *Kv1.3* gene are associated with alterations in glycemic homeostasis and olfactory sensitivity. We evaluated the olfactory performance in 36 healthy subjects and its association with BMI and polymorphism in the human *Kv1.3* gene. Olfactory performance based on the olfactory Threshold, Discrimination and Identification score and their sum (TDI score), was measured using the “Sniffin’ Sticks” Test. Subjects were genotyped for the *rs2821557* polymorphism of the *Kv1.3* gene, whose major allele T was associated with a super-smeller phenotype, lower plasma glucose levels and resistance to diet-induced obesity as compared to the minor allele C. Subjects classified as hyposmic by the total TDI, T and D olfactory scores showed a higher BMI than normosmic ones. Subjects who were TT homozygous or heterozygous exhibited lower BMI and reached higher TDI, T and D olfactory scores than those with CC genotype. These findings show an inverse relationship between olfactory function and BMI, and a significant effect of the *Kv1.3* genotypes on the olfactory function and on the BMI of the subjects.

P2.14

The impact of mitochondrial dysfunction on metabolic and inflammatory profile in an animal model of the autism spectrum disorders (ASD)

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In the last decade, research interest has focused on understanding the etiology and pathogenesis of the autism spectrum disorders (ASD), a developmental disability characterized by impairments in social interactions and repetitive behavioral patterns. Inflammation and mitochondrial dysfunctions in the brain and periphery have been identified as causative factors for impaired cellular functions in ASD, along with a dysregulation of gut-brain crosstalk. Indeed, mitochondria are pivotal mediators of environmental-genetic interactions that regulate healthy metabolism. In this complex framework, the liver is a key organ controlling whole-body inflammatory and metabolic homeostasis. Therefore, we investigated in an animal model of ASD (BTBR mice) the involvement of hepatic mitochondria in the regulation of inflammatory state and hepatic damage. C57Bl/6J and BTBR mice were fed with standard diet, monitoring daily body weights and food intake. Energy balance, inflammation, hepatic mitochondrial function, oxidative stress, and liver histological analyses were assessed. BTBR mice showed increased inflammation and oxidative stress linked to hepatic mitochondrial dysfunction, steatotic hepatocytes, marked mitochondrial fission. Our findings, underscoring the involvement of impaired hepatic metabolism in systemic inflammation of BTBR mice, are of great help in understanding the pathophysiology of ASD, and in developing new diagnostic tools and potential treatment for the disease.

P2.15

Renal physiology in simulated microgravity

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Exposure to microgravity or immobilization results in alterations of renal function, fluid redistribution, and bone loss, which is coupled to a rise of urinary calcium excretion. We provided evidence that high calcium delivery to the collecting duct reduces local Aquaporin 2 (AQP2) mediated water reabsorption under vasopressin action, thus limiting the maximal urinary concentration to reduce calcium saturation. To investigate renal adaptation in microgravity, we investigated the effect of 10 days of continuousbedrest in 10 healthy volunteers. We report here that 10 days of bedrest is associated with a transient significant decrease (day 5) in vasopressin (copeptin) paralleled by a decrease in AQP2 excretion, consistent with an increased central volume to the heart resulting in diuresis. Moreover, bedrest caused a significant increase in calciuria secondary to bone demineralization paralleled by a decrease in PTH. Interestingly, AQP2 excretion inversely correlates with calcium excretion confirming the existence of a local regulatory system to reduce the risk of stone formation. Urinary osteopontin, a glycoprotein exerting a protective effect on stone formation, was significantly reduced during bedrest. Cystatin C, a muscle mass-independent biomarker index of GFR had a clear tendency to decrease during bed rest. We conclude that renal function is altered in simulated microgravity and is associated with an early increase in the risk of stone formation.

P2.16

Sex-based differences in substrate oxidation during moderate-intensity aerobic exercise

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Sex-based differences are well-known to exist in endurance performance, with females generally outperforming males, possibly due to larger lipolytic rates. We performed a meta-analytic and thematic appraisal of sex-based comparative studies on energy substrate utilization during endurance exercise to identify candidate physiological mechanisms accountable for these sex differences. Three databases were searched up to August 2020. Pertinent studies examining substrate utilization during endurance exercise in sedentary/recreationally active and athletic subjects were included. Compared to women, the respiratory exchange ratio was found significantly higher both in sedentary (mean difference, MD: +0.03; $p<0.0001$) and athletic men (MD: +0.02; $p<0.0001$). Greater carbohydrate oxidation was observed both in sedentary (standardized MD, SMD: 0.53; $p=0.006$) and athletic men (SMD: 1.24; $p<0.0001$). Lipidic substrates were oxidized less by sedentary men than women (SMD: -0.77; $p=0.0002$), while no sex differences in fat oxidation were observed in athletes (SMD: 0.06; $p=0.77$). Sex hormones and different patterns of adrenergic activation emerged as the most cited mechanisms thought to mediate the observed sex differences in substrate oxidation.
Our analysis confirmed that men show greater reliance on carbohydrates while women rely more on lipids to sustain endurance exercise. The latter finding was not confirmed in athletes, which is a novel aspect of the present study.

P2.17

Beneficial effects of carvacrol on *in vitro* models of metabolically-associated dysfunctions may be hindered by excess circulating fatty acids interfering with the carvacrol binding to serum albumin

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Carvacrol, a monoterpene phenol of plants, is largely employed as food additive and phytochemical. Our aims were to investigate the beneficial effects of carvacrol using *in vitro* models of hepatic steatosis and endothelial dysfunction, and to verify if and how the binding of carvacrol to albumin, the physiological transporter for drugs in the blood, might be altered by the presence of high levels of fatty acids (FAs) thus impairing the carvacrol bio-distribution *in vivo*. Hepatic FaO cells treated with exogenous FAs mimic hepatosteatosis; endothelial HECV cells exposed to hydrogen peroxide are a model of endothelial dysfunction. In these models, we measured lipid accumulation, free radical production, lipoperoxidation, and nitric oxide release before and after treatment with carvacrol. The carvacrol binding to albumin with/without high levels of FAs was assessed by absorption and emission spectroscopies. Our findings show that *(i)* carvacrol counteracted lipid accumulation and oxidative stress in both hepatocytes and endothelial cells; *(ii)* high levels of FAs reduced the binding of carvacrol to albumin. The beneficial effects of carvacrol on both hepatic and endothelial cells point to its nutraceutical potential. However, high levels of circulating FAs, such those occurring in metabolic disorders, might hinder the carvacrol transport, bio-distribution, and pharmacodynamics.

P2.18

Structure-function relationships in three teleost fish PepT2-type transporters isolated from two teleost fish models, the zebrafish (*Danio rerio*) and the Atlantic salmon (*Salmo salar*)

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The SoLute Carrier 15 (Slc15) family includes H^+-coupled proteins playing a key role in the cellular uptake/reuptake of di/tripeptides, peptide-like drugs and peptidomimetics. In mammals, two transporters, i.e. the low-affinity/high-capacity system Slc15a1 (PepT1) and the high-affinity/low-capacity system Slc15a2 (PepT2), operate in the epithelial cells of intestine and renal tubules. They are expressed also in other epithelial and non-epithelial cells/tissues. In teleost fish, while PepT1-type proteins have been studied in many species, data on PepT2-type are limited to zebrafish (*Danio rerio*) [with one protein functionally characterized, i.e. slc15a2 (pept2); Romano et al. (2006) *Physiol. Genomics* 24(3):207] and, recently, Atlantic salmon (*Salmo salar*) [with two closely related paralogs functionally characterized, i.e. slc15a2a (pept2a) and slc15a2b (pept2b); Vacca et al. *J. Physiol.* (submitted)]. These three proteins fit the high-affinity/low-capacity paradigm defined in the mammalian PepT2-type systems, but comparative analysis of their function highlights peculiarities related to some amino acids (AA) strategically positioned in the sequence. On July 7, 2021 the structure of rat PepT2 was published (www.rcsb.org; PDB Acc. No. 7NQK), offering a new tool to assess association between AA and observed functions in the fish PepT2 proteins. Here, we compare the structural-functional properties of the three fish proteins considering the new hints from the rat PepT2 structure.

P3.1

Study of AQP4 isoforms localization in the olfactory system and their involvement in the olfactory-mediated behavior

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Aquaporin-4 is expressed in two most abundant isoforms, M1 and M23, which aggregates in the plasma membrane of glial cells to form supramolecular assemblies, called orthogonal arrays of particles. Two extended isoforms, M1ex and M23ex, crucial for AQP4 anchoring at the BBB level were recently discovered. In the present study we evaluate the contribution of the different AQP4 isoforms in the olfactory system by using two mouse models, respectively missing the AQP4ex and the M23 isoforms. Immunofluorescence experiments showed AQP4 and AQP4ex expression in the basolateral membrane of supporting cells of the olfactory epithelium (OE) in the control mice. In the AQP4M23-KO, most of the AQP4 signal was lost indicating the major contribution of the M23 isoform in the AQP4 plasma membrane assembly as it occurs in other CNS regions. In the olfactory olfactory bulb (OB), AQP4 and its extended isoforms revealed an astrocytic perivascular expression pattern and a sparse reticular one in the glomerular layer. To better understand the role of AQP4 isoforms in the olfactory sensation, we performed the "buried cookie test", where the latency to find the cookie was recorded. Surprisingly, AQP4M23-KO were two fold faster in retrieving the cookie, showing greater olfactory abilities, than control and AQP4ex-KO. These data suggest that AQP4 may play an important role in generating and maintaining the OE microenvironment necessary for olfactory sensitivity.

P3.2

Target selection, but not distractor suppression, is impaired by single pulse TMS over right Frontal Eye Field

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The ability to suppress irrelevant but attention-grabbing information while executing a task is a cardinal function of the frontoparietal dorsal attention network that underlies goal-directed control in human behaviour. Here, to scrutinize the role of the right Frontal Eye Field (FEF) in selection and filtering, we sought to investigate the temporal contribution of this critical node within such network by means of single pulse Transcranial Magnetic Stimulation (spTMS). We interfered at 3 different time points (0, 100, 250 ms after array onset) with the function of right FEF in healthy participants performing a visual search task. Participants were asked to discriminate the orientation of a target while ignoring a salient distractor, when present. The results did not reveal a varying effect for the different time points. Crucially, stimulation of the right FEF significantly prolonged RTs, but only when the target was located in the hemifield contralateral to the active stimulation site. Moreover, right FEF stimulation did not produce any effect over the cost engendered by the salient distractor, irrespective of both target and distractor location. These findings indicate that the right FEF plays a critical role in orienting to - and selection of - the target stimulus, especially for the hemifield contralateral to the stimulation site. Further research is needed in order to decipher the precise role of the right (and left) FEF in target selection and distractor filtering.

P3.3

Brain repair in temporal lobe epilepsy: an *in vivo* investigation

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Temporal lobe epilepsy (TLE) is an adulthood focal epilepsy often refractory to anti-epileptic medications, and Mesial TLE (MTLE) is its most severe form mainly characterized by hippocampal sclerosis. Intracerebral transplantation of neural stem cells (NSCs) is evolving as an attractive therapy for promoting regeneration and repair in various brain disorders including TLE. However, hostile environment makes difficult NSCs survival. In this regard, polymer carriers for cell delivery have been proposed to promote NSCs survival, differentiation and maturation.
Our aim was to evaluate whether NSCs survival in a hostile condition may be preserved by co-injection with the algal polysaccharide alginate.
Adult male Sprague Dawley rats were treated with pilocarpine to induce status epilepticus (SE), a brain insult leading to TLE. Two weeks after SE, rats were bilaterally injected with the cytotoxic agent ibotenic acid in ventral CA3 area, and 4 days later received infusion of NSCs and alginate. We assessed alginate-mediated cell survival via immunofluorescence performed at different time points after cell and matrix infusion.

P3.4

Is Betaine a substrate also for GABA transporter 1 (GAT1)?

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The re-uptake of the inhibitory neurotransmitter, ɣ-aminobutyric acid (GABA) in the central nervous system (CNS) is the primary function of the sodium- and chloride dependent GABA transporters. Among all four GABA transporters (GAT1-3 and BGT1), GAT1 is extensively investigated, as it is considered the primary regulator of GABA in the CNS. After GAT1, BGT1 (betaine/GABA transporter) has the most affinity for GABA, but still its physiological role in the brain is unclear and debatable. The nervous tissues can not only accumulate high concentrations of betaine (N-trimethylglycine), but also selectively prefer it over other osmolytes e.g., myo-inositol, creatine. Some recent studies in *C. elegans* show betaine regulated ion channels in the nervous system. Betaine is also being considered as treatment for schizophrenia. Our electrophysiological experiments on *X. laevis* oocytes expressing rGAT1, show the presence of inward transport currents in the presence of betaine. Despite acting like a substrate, when betaine is present, the changes in membrane voltages still elicited pre-steady state currents indicating a different transporter interaction mechanism than that of GABA. In competitive assay GABA and Betaine showed neither competition nor cooperation between them. Our preliminary results indicate betaine induced transport-like currents in GAT1. An actual influx of betaine needs to be confirmed, to understand the different behaviour of GAT1 in the presence of the two substrates.

P3.5

Movement restraint alters neural stem cells metabolism

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Adult neurogenesis happens in the mammal brain although restricted to few areas. The synergistic action of extrinsic and intrinsic factors in the microenvironment of these areas controls the fate of the Neural Stem Cells (NSCs) and is able to adjust the balance between undifferentiated progenitor cells and newly differentiated cells. Within extrinsic factors, the alteration of movement activity plays a pivotal role.
Indeed, some neurological diseases are associated with, or are the cause of, movement impairments; among them, spinal cord injury and spinal muscular atrophy are examples with analogous effects on anti-gravity muscles. Similarly, it is known that prolonged space missions induced functional alterations in the central nervous system (CNS).
Low levels of exercise represent a major risk factor of developing metabolic alteration that could affect the CNS.
In previous work, we determined that NSCs obtained from suspended animals (hind limb unloading model (HU)) had different neurogenic characteristics.
NSCs obtained from HU or naive mice were studied for their metabolic properties in particular for their capacity to uptake glucose, their level of ATP, and their ROS production.
All the results indicated that HU-NSCs had some impairments in their metabolism compared with CTR-NSCs.
A better understanding of the effects of motor deprivation on neurogenesis is relevant as it can open new avenues to design effective treatments coupled with rehabilitation/training interventions.

P3.6

An imbalance in autophagy contributes to retinal damage in the oxygen-induced retinopathy rat model

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In retinopathy of prematurity (ROP), oxygen fluctuations represent a stressful condition resulting in retinal cell dysfunction and death. As a compensatory response, the activation of autophagy could support retinal cells in maintaining their viability under altered oxygen tension. However, an imbalance in the autophagic mechanisms could be detrimental, amplifying the induction of retinal cell death pathways and the exacerbation of the disease. Here, a rat model of oxygen-induced retinopathy (OIR), a recognized model of ROP, was used to investigate a possible alteration in autophagy and a putative relationship between autophagic mechanisms and cell death in the rat retina. Western blot and immunofluorescence analysis demonstrated a dysregulation of autophagy in the retina of OIR rats, with a contemporary activation of retinal apoptosis and necroptosis. Moreover, we found that the treatment with 3-methyladenine (3-MA), an autophagy blocker, inhibited the OIR-dependent increase in autophagy and necroptosis, whereas no evident effect on apoptotic markers was found. However, retinal function resulted still compromised in this model, even after 3-MA treatment. Taken together, these results indicate an involvement of autophagy in the pathogenesis of ROP, suggesting a novel molecular target against the disease.

P3.7

Stable and dynamic response population coding in prefrontal cortex

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Prefrontal cortex (PFC) plays a key role in several goal-directed processes. PFC cells encode response choices across periods that require future actions to be kept in memory until their execution and monitor their outcome after execution. Great attention has been given to characterize how single cells interact to represent information across time and cognitive processes according to a stable or dynamic scheme. Nevertheless, little is still known about the specificity of these schemes for distinct cognitive processes and whether different PFC areas might represent the same information adopting different schemes. We investigated the response population coding proprieties of Dorsolateral (PFdl), Orbital (Fpo) and Frontopolar (PFp) prefrontal cortex across different task epochs of a rule-based task. In this task, two rhesus monkeys were trained to make a saccadic response toward one of two peripheral targets according to a specific rule associated with a visual cue. We focused our analysis on the delay period (DP), where the response is generated and maintained in memory and the feedback period (FP), where the monkey has reached the selected target waiting to know the outcome of his choice. We observed that PFdl encodes response during DP and FP with distinct coding schemes: static during the DP and dynamic in FP. A comparison of population coding during FP highlighted that the coding scheme was dynamic in PFdl and PFo compared to PFp, where the representation was static.

P3.8

Cognitive and visual function, a simultaneous decline in neuroinflammation

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The earliest phase in most of the neurodegenerative diseases is an undercurrent and chronic neuroinflammation whose mechanisms and mediators might be shared with the specific pathogenesis. Despite most of neurodegenerative diseases have no cure, an early diagnosis might improve therapeutic outcomes slowing down the disease. Unfortunately, this preliminary neuroinflammatory stage is not easily identifiable, especially in the brain. One of the most accessible and sensitive part of the CNS is the retina which might play a reporter role on brain healthiness. However, specific correlation between visual function and neuroinflammation is not completely understood. Here we characterize a murine model of lipopolysaccharide (LPS)- induced neuroinflammation in order to identify functional changes of the retina which can be predictive for an early diagnosis of brain dysfunctions. Adult mice were intraperitoneally injected for 5 days with 0.25 mg/Kg of LPS. Electroretinogram (ERG) recordings and novel object recognition test (NOR) were performed at different time points up to 10 days from the last injection. ERG analysis describes a severe decline in retinal response at each time points. Analogously, NOR shows a significant cognitive impairment with no recovery up to 10 days. These results show a link between cognitive decay and retinal function in neuroinflammatory conditions confirming the essential role of the retina as a window of the brain in pathological conditions.

P3.9

Long-term behavioral and histological profile following CA3 ventral lesion

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Ventral hippocampus relates with emotional processing and stress responses, including anxiety and depression-like behavior. Lesions in the ventral CA3 region, specifically, are a hallmark of mesial temporal lobe epilepsy (MTLE), and may cause psychiatric co-morbidities often observed in MTLE patients. We aimed to evaluate the long-term effects of a ventral CA3 lesion induced by the cytotoxic compound ibotenic acid.
Ibotenic acid was bilaterally injected into ventral CA3 of adult male Sprague Dawley rats. Sham-operated animals underwent the same surgery, including needle insertion, without injection of the cytotoxic agent, and served as controls. A battery of behavioral tests, including the open field and the novel object location tests, the elevated plus maze, the forced swimming test and the resident-intruder paradigm have been employed, followed by brain collection for qualitative and quantitative histological analysis.
Behavioral performances were not altered in lesioned animals compared to sham rats 12 weeks post injection, although cell loss with a consistent glial scar formation was verified by immunohistochemistry and immunofluorescence studies. Our results suggest two possible interpretations: the lesion induced by ibotenic acid is too small to affect behavioral performance at the tests we have carried out, or 12 weeks is a time long enough to allow some compensatory phenomena. This remains an open question that deserves further investigations.

P3.10

Sex affects resting state alpha electroencephalographic rhythms in cognitively unimpaired seniors and patients with Alzheimer's disease and amnesic mild cognitive impairment

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It is well-known that the resting-state eyes closed electroencephalographic (rsEEG) rhythms show the highest amplitude in the posterior regions at the alpha (8-13 Hz) frequencies, as a reflection of a cortical inhibition located in posterior visual and visuospatial cortical areas during a state of deprivation from visual stimuli. Here, we tested the hypothesis that sex may affect cortical sources of alpha rhythms in normal elderly (Nold) seniors and patients with Alzheimer's disease and mild cognitive impairment (ADMCI).
Datasets in 69 ADMCI and 57 Nold individuals were taken from an international archive. The rsEEG rhythms were investigated at individual delta, theta, and alpha frequency bands and fixed beta and gamma bands. Each group was stratified into matched females and males.
The sex factor affected the magnitude of rsEEG source activities in the Nold seniors. Compared to the males, the females showed greater alpha source activities in all cortical regions. Similarly, the posterior alpha source activities were greater in the ADMCI females than the males. Notably, the present sex effects did not depend on genetic, neuropathological, structural, and cerebrovascular variables characterizing sporadic AD-related processes.
These results suggest the sex factor may significantly affect neurophysiological brain neural oscillatory synchronization mechanisms underpinning the generation of dominant rsEEG alpha rhythms to regulate cortical arousal during quiet vigilance.

P3.11

Combined dopaminergic and gabaergic regulation of prefrontal cortex activity by the cerebellum in anesthetized mice

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Alterations in cerebello-prefrontal cortex (mPFC) connections characterize several cognitive dysfunctions, suggesting that the cerebellum might have a crucial impact on mPFC proper functioning. To investigate this connection, we used single-unit recordings *in vivo* in the prelimbic area (PrL) of anesthetized mice. Electrical stimulation of the contralateral cerebellar dentate nucleus mainly elicited a pause in PrL neurons firing, sometimes followed by an excitation rebound. To investigate the nature of PrL responses, we co-applicated selective dopamine D1-like and D2-like receptor antagonists (SCH23390 and sulpiride, respectively), as well as GABAA receptor antagonist (gabazine). The blockade of dopaminergic transmission modulated PrL neurons spontaneous firing rate without abolishing pause responses, which were suppressed by gabazine perfusion, suggesting a prominent role of local inhibition. Interestingly, gabazine perfusion following dopamine receptors antagonists showed different effects, hinting for dopaminergic and GABAergic interplay in controlling PrL neurons activity. Our data show that cerebellar activation silences PrL activity involving local inhibitory circuits (likely acting through thalamic projections). PrL neurons activity is modulated by the dopaminergic system through mechanisms that influence but do not occlude GABAergic control. Overall, these findings provide evidence for a complex cerebellar functional control over the PrL.

P3.12

The Ts65Dn mouse model of DS shows dendritic alterations in neocortical pyramidal neurons at early life stages

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Down syndrome (DS) is a genetic condition due to triplication of chromosome 21 and is associated with intellectual disability (ID). ID represents the most invalidating aspect of DS and can be mainly attributable to neurogenesis impairment and dendritic pathology. These defects are replicated in the adult Ts65Dn mouse, the most used model of DS. No information, however, is available regarding the ontogeny of dendritic alterations in this model. In the present study, we used pups of Ts65Dn mice to establish whether early dendritic alterations are present, analogous to those that characterize neonates with DS. To this purpose, in Ts65Dn mice aged 2 days we measured the dendritic trees of Golgi-stained pyramidal neurons located in layer II/III of the frontal cortex.
An evaluation of the length and number of branches of the apical dendritic tree showed no alterations. We found, however, a moderate hypotrophy of the apical collateral dendrites and a larger hypotrophy of the basal dendritic tree, with absence of higher order branches. In the pyramidal neurons of P2 mice, there were no dendritic spines but only somatic spines. Analysis of the latter showed no differences between Ts65Dn and euploid mice.
The absence of a severe dendritic pathology in Ts65Dn pups is comparable to the ontogeny of dendritic alterations in children with DS. These results suggest that the Ts65Dn model can be used to study the causes of dendritic alterations in DS and to devise possible preventive treatments.

P3.13

Androgen receptor with an elongated polyglutamine tract dysregulates muscle expression of myo-miRs and of their target genes

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The elongation of the polyQ tract in the Androgen Receptor (AR) is responsible for Spinal and Bulbar Muscular Atrophy (SBMA), a neurodegenerative disease involving lower motor neurons. Patients generally present skeletal muscle atrophy with mixed myopathic alterations as well as neurogenic features of denervation in muscle biopsy. Recent studies suggested that muscle degeneration is not only due to motor neuron death, but intrinsic changes of affected muscle cells may be primarily responsible for disease onset and/or progression. Furthermore, the conditional deletion of peripheral polyQ-AR counteracts the disease. Notably, almost all aspects of skeletal
muscle development/regeneration are regulated by miRNAs, and specifically by myomiRs that are
selectively enriched in muscle tissue. In this study, we analyzed by RT-qPCR the expression of several myomiRs crucial for muscle development/regeneration (miR-206, miR-133a, miR-133b, and miR-1) along with some of their putative target genes (*Pax7*, *Myog*, *Myod1* and *Mef2a*) in the skeletal muscle tissue of a knock-in SBMA mouse model at different disease stages. We confirmed the results by western blot analyses and extended the observations to the serum of human patients. Obtained data suggest that a potential regenerative response in muscle tissue of SBMA animals may be triggered during disease progression, but the dysregulation of specific myomiR/target
gene pairs may account for muscle impairment and inefficient repair mechanisms.

P3.14

Role of endocannabinoids and TRPV1 channels in the bioelectric activity of hippocampal neurons

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It has been reported that endocannabinoid system is an important player in the regulation of neuronal bioelectrical activity, relying on receptor-mediated mechanisms. Amongst these, Cannabinoid receptor type 1 (CB1r) and Transient Receptor Potential Vanilloid type 1 (TRPV1) are both modulated by endocannabinoids, involved in the transduction of stimuli in the pre-synaptic neuron and prompt downstream pathways in the post-synaptic neuron. To investigate the role of CB1r/TRPV1 interplay, we applied whole-cell patch clamp technique to visualize the eventual variations in terms of membrane current and action potentials induced by pharmacological manipulation in rat hippocampal neurons. We modulated the activity of the CB1r and TRPV1 exploiting anandamide (AEA), CB1r and TRPV1 agonist, capsaicin (CAP), a TRPV1 agonist and capsazepine (CPZ), a TRPV1 antagonist. Our data show that AEA influences steady membrane current with respect to controls. Furthermore, drug application significantly modifies action potentials amplitude, duration and frequency. In particular, the co-treatment of AEA and CPZ increases the amplitude of action potentials, reduces their duration and thus increases their frequency. These preliminary results support the involvement of TRPV1 in the cannabinoid modulation of the bioelectrical activity in rat hippocampal neurons. Indeed, the concurrent blockade of these channels and activation of CBr influences basic properties of neuronal function.

P3.15

Role of the neurovascular unit (NVU) and of the redox state in the amyotrophic lateral sclerosis pathogenesis

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Background Oxidative stress and alteration of mitochondrial function and of the neurovascular unit (NVU) could play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS). **Aim**: We analyzed the redox system in plasma of ALS patients and its effect on oxidative stress/mitochondrial function in human umbilical cord-derived endothelial vascular cells (HUVEC) and astrocytes. The role of riluzole/acetyl L carnitine (ALCAR) has been addressed, too. **Materials and Methods** The study was conducted on 25 ALS patients belonging to the Neurological Clinic of Novara, compared with a control group. In blood samples, Thiobarbituric Acid Reactive Substances (TBARS), glutathione (GSH) and nitric oxide (NO) were measured through specific assays. *In vitro* the effects of plasma on mitochondrial reactive oxygen species (mitoROS) release, NO release, cell viability, mitochondrial membrane potential were examined in HUVEC/astrocytes. **RESULTS** We found an increase in TBARS and a reduction of GSH and of NO in plasma of ALS patients. In vitro, an increase of mitoROS and a decrease of cell viability and mitochondrial membrane potential were found in both HUVEC and astrocytes treated with ALS plasma. In HUVEC, NO release was reduced, as well. The above variables were modulated by riluzole/ALCAR. **CONCLUSIONS** The results obtained highlight the central role exerted by oxidative stress and NVU in ALS and suggest how unknown plasma factors could be involved in the pathogenesis of the disease.

P3.16

Cerebellar climbing fibers can undergo activity-dependent structural plasticity

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The structure and function of neuronal circuits can be modified by experience during the encoding of memories, or under pathological conditions. Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. These fibers originate in neurons of the inferior olivary (IO) nucleus, in the brainstem, and can undergo dramatic structural modifications after lesions. Several lines of evidence suggest that structural plasticity may be induced by either a blockade or an increase of neuronal firing and that it may rely on the growth-associated protein 43 (GAP-43) that is highly expressed in these fibers. However, the dependence on intrinsic excitability and the extent of such plastic events are largely unclear.
Here we investigate how modifications of the CF intrinsic excitability affect their structure and the physiology of the olivo-cerebellar circuit. To do this, we chronically altered CF intrinsic excitability and tonic firing by in vivo knocking-down or knocking-out voltage-gated sodium channels or other proteins involved in the regulation of intrinsic excitability and reactive plasticity at the inferior olive of adult mice. We analyzed CF 3D morphology, presynaptic terminals and postsynaptic spines, as well as the functional consequences in CF synaptic transmission. We show that activity-dependent structural plasticity can occur at CFs and PCs, potentially affecting cerebellar function.

P3.17

Copper-mediated PLK2 protein modulation in α-synuclein phosphorylation process in Parkinson’s disease

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Copper (Cu) homeostasis is a key element for human beings. Deficiencies lead to the formation of unfunctional apoproteins; increases in its concentrations lead to oxidative stress and alterations in many body compartments, primarily the nervous system. Neurodegenerative diseases, such as Parkinson's disease (PD), are widely linked to alterations in Cu ions levels, which trigger protein aggregation phenomena through ROS production, altering degradation processes and acting themselves as nucleation centers. The hallmark of PD are Lewy’s Bodies (LBs), neuronal formations whose main component is represented by α-synuclein protein. In LBs the protein is extensively phosphorylated on a Serine in position 129, whereas physiologically this post-translational modification (PTM) is only minimally detectable. This observation shed light on such a PTM, encouraging investigations concerning the main kinases involved in the phosphorylation process, their potential contribution to the pathology, and the role of Cu in modulating their activity. Our observations in human differentiated SHSY5Y neuroblastoma cells have shown a Cu dependent modulation of PLK2 kinase. The identification of an axis between an intracellular increase in Cu and pSer129 α-synuclein levels, strengthens the evidence relating the metal dyshomeostasis to the earliest phases of PD onset. This opens up avenues for therapeutic intervention at this level.

P3.18

Misophonia: analysis of the neuroanatomic patterns at the basis of vegetative and psychiatric symptoms

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Background Misophonia is a condition characterized by intense emotional reactions and vegetative symptoms in response to “trigger” sounds. To date, knowledge about misophonia is scarce and solid scientific evidence would be required to better characterize it. **Aim** We aimed to evaluate the vegetative response to “trigger sounds”, psychometric assessment and the neurological pathways in misophonics. **Materials and Methods.** Seven “trigger sounds” were applied to 11 misophonic subjects and 44 healthy controls. The effects were examined on functional Resonance Magnetic Imaging (fMRI), heart rate variability (HRV) and galvanic skin conductance (GSC). Psychometric assessment investigated the misophonic construct, obsessive, anxiety and depressive symptoms, resilience, anger and motivation. **Results.** The misophonics shown increased pattern of sympathetic activation, both at HRV and GSC analysis. At fMRI, temporal cortex, hippocampus, insula, ventromedial prefrontal cortex (vPFC), cingulate cortex, cerebellum and premotor cortex were found to be activated. The psychometric assessment evidenced a misophonia construct and low resilience in the misophonics in the absence of other psychopathological symptoms. C**onclusions.** The results of our study suggest the involvement of a specific vPFC-auditory-insula-limbic pathway at the basis of the sympathetic activation and psychometric assessment in the misophonics. These results could help to better classify this disorder.

P3.19

$Ca_V3.2$ channel as a keystone to clear up differential Bortezomib and Carfilzomib neurotoxicity

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Proteasome inhibitors (PIs) represent the gold standard in the treatment of multiple myeloma. Among PIs, Bortezomib (BTZ) is used as first line therapy, but causes peripheral neuropathy (PN), approximately in 50% of patients. Carfilzomib (CFZ), a second-generation PI, induces a significantly less severe PN. The underlying mechanisms of chemotherapy-induced peripheral neuropathy (CIPN) is still poorly understood. Accumulating evidence regarding CIPN suggests the involvement of multiple mechanisms, independent of their antineoplastic action like changes in cytoskeleton and altered expression and/or function of ion channels. T-type calcium 3.2 channel ($Ca_v3.2$), which is expressed in sensory neurons and is responsible for 80% of the T type calcium current, is considered the most important target involved in neuropathic pain. A possible involvement of calcium currents in BTZ and CFZ neurotoxicity was studied in an *in vitro* model of primary cultures of adult mice dorsal root ganglia (DRG). High Voltage Activated (HVA) and Low Voltage Activated (LVA) currents were recorded by using whole-cell patch-clamp techniques in DRG neurons after 16 hours of treatment with BTZ and CFZ at a concentration of 2.8nM 3.2nM, respectively. Our results suggest that the incubation with BTZ had a preferential effect on the LVA current increasing its amplitude, while CFZ showed a trend in decreasing both LVA and HVA calcium currents.

P3.20

Imbalance in Angiotensin II/Angiotensin 1-7 system affects pial microcirculation during rat brain hypoperfusion and reperfusion

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The present study was aimed to *in vivo* compare the effects induced by Angiotensin II (Ang II, generated from Ang I by angiotensin converting enzyme 1: ACE1) and Angiotensin-1-7 (Ang 1-7, generated from AngII by angiotensin converting enzyme 2: ACE2) on rat pial microcirculation during brain hypoperfusion and reperfusion injury. To quantify the microvascular parameters during hypoperfusion and reperfusion we used a fluorescence microscopy technique.
Rats treated with AngII, administered prior to hypoperfusion and at the beginning of reperfusion, showed an exacerbated microvascular damage with stronger vasoconstriction in order 3 vessel diameter (-20.5±1.8% vs. -15.7±2.1% of baseline), increase in leakage (evaluated by normalized grey levels was 0.53±0.02 vs. 0.46±0.03 of baseline), higher decrease in capillary perfusion (-64±3% vs. -52±2% of baseline) and marked reactive oxygen species formation compared to the values detected in hypoperfused animals. The candesartan cilexetil (AT_1R antagonist) administration prior to AngII prevented the microvascular damages AngII-induced. Rats treated with Ang 1-7 resulted protected from hypoperfusion/reperfusion damage.
In conclusion, under hypoperfusion/reperfusion conditions AngII and Ang 1-7 are effective in inducing opposite effects on microvascular networks. Therefore, when ACE1 activity is predominant with consequent Ang II prevalence AT_1R is activated and promotes redox stress with successive vascular damage and organ implication.

P3.21

Age affects resting state alpha electroencephalographic rhythms in cognitively unimpaired seniors and patients with Alzheimer's disease and amnesic mild cognitive impairment

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The resting-state eyes closed electroencephalographic (rsEEG) alpha (8-12 Hz) rhythms reflect cortical neural synchronization mechanisms underpinning the inhibition of sensory, cognitive, and motor areas in parietal, temporal, and occipital cortex during a condition of low vigilance. Here we tested the hypothesis that age may diversely affect rsEEG alpha rhythms recorded in normal elderly (Nold) seniors and patients with Alzheimer's disease and mild cognitive impairment (ADMCI).
Clinical and rsEEG datasets in 63 ADMCI and 60 Nold individuals were taken from an international archive. The rsEEG rhythms were investigated at individual delta, theta, and alpha frequency bands, as well as fixed beta and gamma bands. Each group was stratified into three subgroups based on age ranges.
Compared to the younger Nold subgroups, the older one showed greater reductions in the rsEEG alpha rhythms with major topographical effects in posterior regions. On the contrary, in relation to the younger ADMCI subgroups, the older one displayed a lesser reduction in those rhythms. Notably, the ADMCI subgroups pointed to similar cerebrospinal fluid AD diagnostic biomarkers, gray and white matter brain lesions, clinical and neuropsychological scores.
The present results suggest that age may represent a deranging factor for dominant rsEEG alpha rhythms in Nold seniors, while rsEEG alpha rhythms in ADMCI patients may be more affected by the disease variants related to earlier vs later onset of the AD.

P3.22

Motor and perceptual aspects of face emotional expressions in young and aged subjects

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Previous works suggested that not only occipito-temporal areas but also the primary motor cortex (M1) are engaged in the recognition of faces expressing emotions. It is known that aging impairs the ability to recognize face emotional expressions, but no studies have investigated the possible role of face M1 in this impairment. This work investigated the effects of the passive viewing of face emotional expressions on perceptive brain regions and on M1 innervating lower face muscles. Young (n=17: age 24.41±0.71 y.o) and aged subjects (n=17: age 63.82±0.99 y.o) underwent assessment of *i*) event related potentials (P100 and N170 waves) from occipito-temporal areas, *ii*) motor evoked potentials from the depressor anguli oris muscle (short-latency intracortical inhibition, SICI, and intracortical facilitation, ICF) and *iii*) recognition task after presentation of images reporting happy, sad and neutral faces. Compared with young subjects, the aged group showed a delayed N170 wave and a smaller P100 wave following the view of sad but not happy or neutral expressions, along with less accuracy and longer reaction times in the recognition task. Aged subjects presented less SICI than young subjects, but facial expressions of happiness decreased SICI and increased ICF in face M1, with no differences between groups. Data suggest that aging impairs visual attention for sad face expressions, while perception of happiness and its excitatory effects on face M1 remain preserved.

P3.23

Formation of cell assemblies in the prefrontal cortex of macaque monkeys during a distance and a temporal discrimination task

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Neurons synchronize their activity organizing into larger groups called cell assemblies. We studied the formation of cell assemblies in the macaque prefrontal cortex during the execution of two tasks involving duration and distance discriminations. Using a recent algorithm for cell assembly detection, we extracted the spikes fired during the assembly activation (assembly-spikes) from the overall activities (all-spikes) and we calculated their optimal bins and lags. Focusing on assembly of two cells, we found that the formation of such assemblies occurred more frequently for close neurons (neurons recorded by the same electrode) than for farther ones. We found also that their synchronization occurred without any lag more often for close than for far neurons. Therefore, distant neurons appear to have activities that correlate more sequentially. Furthermore, we observed that these assemblies of neighboring neurons persisted more often between tasks suggesting that the assemblies formed by neighboring neurons were more multipurpose. Finally, we focused on the spatial preferences for the right and the left responses. We found that while the probability that both neurons shared the same preference was close to chance considering the all-spikes, it increased to 80% by considering only the assembly-spikes. Our results suggest that this method of analysis has great potential for getting an insight into how neurons of the assembly orchestrate during task execution.

P3.24

LRRK2 phosphorylation on serine 935 impacts on synaptic vesicle trafficking

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Leucine-rich repeat kinase 2 (LRRK2) is a widely expressed serine-threonine kinase involved in the control of vesicular trafficking and associated with autosomal dominant Parkinson's disease. LRRK2 activity is tightly regulated by the balance between auto- and hetero-phosphorylation of the protein, but how this complex regulation relates to molecular and cellular features of LRRK2 is unclear. Looking at the distribution of LRRK2 phosphorylated residues, most interest has focussed on the serine 910/935. In this study, we investigated the functional role of LRRK2 phosphorylation at Ser935, using alteration of synaptic vesicles (SV) trafficking as a read-out of LRRK2 activity. By combining total internal reflection fluorescence microscopy (TIRFM) and synaptopHluorine, a genetically encoded sensor of vesicles fusion and recycling, we monitored SV trafficking in the neuroblastoma cell lines (N2a and SH-SY5Y) expressing the LRRK2 full-length protein (WT) or the S935A phospho-death mutant. We found that the number of spontaneous fusion events and total fluorescence elicited under basal conditions was significantly increased upon over-expression of the S935A variant. Interestingly, immunofluorescence and TIRFM studies demonstrated different subcellular localization of LRRK2 WT and S935A mutant. We also identified signalling pathways involved in LRRK2 heterophosphorylation. These findings may have important implications for understanding the pathophysiological mechanisms of LRRK2.

P3.25

Biophysically realistic computational models of mouse and human Purkinje cells

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Purkinje cells (PC) are renowned to be the one of the most complex neurons of the brain and host complex synaptic, biochemical and biophysical machineries including ionic channels, synaptic receptors and Ca^{2+} buffering systems. Ca^{2+} channels in the dendrites and Na^{+} channels in the Axon Initial Segment (AIS) proved critical to generate the rich excitable properties of these neurons. To study the impact of 3D neuronal morphology on intrinsic and synaptic responsiveness, we combined anatomical reconstructions of 19 mice and 6 human PCs with electrophysiological recordings and computational modeling. Human PCs showed 3-12 times more extended dendrites with increased complexity but similar fractality index. In both species, the models were all endowed with ionic channels maintaining the same distribution validated previously (Masoli and D'Angelo, 2017) and were optimized against electrophysiological templates. All models showed similar spontaneous firing and responses to positive and negative current injection. Moreover, synaptic stimulation showed that 50 synapses were similarly capable of generating the typical burst/pause response pattern in both species. Typically, the human PC had at least 20 more independent computational compartments than mice PCs. Therefore, these results suggest that during the course of evolution, the complexity of the human PC structure provided a stronger enhancement in computational capabilities compared to the mouse.

P3.26

Activation of human microglia clone 3 (HMC3) in response to stress conditions: are long non-coding RNAs involved?

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Microglia are the resident macrophages of the central nervous system and their role in inflammation is related to their phenotypes: i) anti-inflammatory M2 characterized by the increase of the phagocytic activity and the release of neuroprotective factors; ii) pro-inflammatory M1 characterized by the release of cytotoxic factors.
It has recently been observed that the modulation of microglia phenotype in neurodegenerative diseases might be triggered by long non-coding RNAs (lncRNAs), a class of RNA molecules dysregulated in many neuropathologies. Meta-analysis studies identified the differential expression of lncRNAs in PD brain *vs* control and also in both human neuroblastoma SH-SY5Y cells and human monocytic THP-1 cells activated with 6-OHDA or LPS. To clarify their role also in microglia under stress conditions, we enrolled the human microglia cell line HMC3 to overcome the evolutionary conservation problem of lncRNAs among different species. To setup different models of activation, we exploited a treatment with IFN-γ boosted by glucose to promote the NFK-β inflammatory pathway and a Parkinson's-related model with 6-OHDA treatment. For the first time, we detected the increase in M1-marker expression (iNOS) in HMC3 cell by both activation stimuli. In addition, a slight phagocytic activity was preserved in the Parkinson's model attributable to an early-stage model of disease. Now a functional study of specific lncRNAs according to the different stimuli is on-going.

P3.27

Histone acetylation defects in brain precursor cells: a potential pathogenic mechanism of AGC1 deficiency proliferation/differentiation dysfunction

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AGC1 deficiency is an ultra-rare genetic disease characterized by hypomyelination and brain atrophy, caused by mutations in SLC25A12 gene, leading to a reduction in mitochondrial aspartate-glutamate carrier 1 (AGC1) activity. In neuronal and oligodendrocytes precursor cells (NPCs and OPCs), it reduces proliferation and accelerates OPCs differentiation, with gene expression alerations. Epigenetic regulation of gene expression through histone acetylation plays a crucial role in this process and is modulated by mitochondrial metabolism. Histones acetylation dysfunctions have been dissected in *in vitro* models of AGC1 deficiency OPCs and NPCs, showing an altered expression of transcription factors involved in brain precursor cells proliferation/differentiation, a reduction in histone acetylation with parallel changes in histone acetyltransferase (HAT) and deacetylases (HDACs) expression and activity. HATs inhibition through curcumin stimulates the differentiation of OPCs, while HDACs inhibition through SAHA has a limited effect on proliferation, but it stimulates OPCs differentiation. In NSCs, both treatments affect the commitment towards glial cells. These data contribute to clarify the molecular and epigenetic mechanisms regulating OPCs and NPCs proliferation/differentiation in order to identify new potential targets for therapeutic approaches able to increase the OPCs pool and to sustain their differentiation towards oligodendrocytes and therefore myelination/remyelination.

P3.28

The splanchnic anti-inflammatory pathway dampens down the inflammatory response induced by different immune challenges in mice

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The splanchnic anti-inflammatory pathway (SAIP), the efferent arm of the inflammatory reflex, inhibits the inflammatory response in endotoxemic rats. In this study, we investigated the involvement of the SAIP in the reflex control of inflammation in a new species, the mouse, challenged with different TLRs agonists: Lipopolysaccharide (LPS, TLR-4 agonist), Polyinosinic:polycytidylic acid (Poly I:C, TLR-3 agonist), and dipalmitoyl-S-glyceryl cysteine (Pam2cys, TLR-2 and 6 agonist).
Forty-seven C57BL6 mice were subjected to bilateral splanchnic nerves section, to disengage the SAIP, or sham surgery prior to the i.v. administration of one of these inflammatory stimuli: LPS (60µg/kg), Pam2cys (34µg/kg) or Poly:IC (1 mg/kg). Ninety minutes after the i.v. injection, 0.5ml of blood was collected via intracardiac puncture for subsequent cytokines measurement.
A consistent pattern emerged in response to the three different challenges: Tumor necrosis factor (TNF) and Monocyte Chemoattractant Protein-1 (MCP-1) levels were raised by prior splanchnic nerve section, while levels of the anti-inflammatory cytokine interleukin 10 (IL-10) were reduced. The raised TNF:IL-10 ratio after splanchnic nerve section indicates an enhanced inflammatory state when the SAIP is disabled.
In conclusion, we demonstrated that, in mice, the reflex activation of the SAIP drives a coordinated anti-inflammatory action in response to different immune challenges acting on different TLRs.

P3.29

High-density multielectrode array recordings reveal computational complexity in cerebellar cortical processing

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The cerebellar cortex has a prominent forward architecture that was used to hint for a low complexity of the observed spatial-temporal activities with respect to the cerebral cortex. However, the complexity of the cerebellar responses has never been assessed experimentally. We quantified the *complexity* of the spatial-temporal responses to mossy-fiber (MF) stimulation in cerebellar slices with the Perturbational Complexity Index (PCI), an index which has been used to summarize the richness of TMS/EEG spatial-temporal patterns in conscious/unconscious human subjects. We calculated PCI applied to high-density multielectrode array (hdMEA) recordings in acute cerebellar slices, which allowed us to measure the spatiotemporal interaction between granule cell (GrC) and Purkinje Cell (PC) after MFs stimulation at different frequencies (from single shock to 100 Hz). MF stimulation triggered local field potentials in the granular layer and single unit frequency changes in PCs generating typical spatiotemporal patterns. Single-shock stimuli evoked significant activities for ~20 ms in the granular layer and little activation of the down-stream Purkinje layer. The analysis showed that PCI nearly doubled with frequency, more evidently in the coronal than sagittal plane, probably reflecting the intense spread of activity along the parallel fibers. These results indicate that complexity in the cerebellar circuit changes dynamically with input frequency and the orientation of signal flow.

P3.30

Sleep-related effects on the degree of Tau protein phosphorylation following the induction of synthetic torpor in the rat

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Synthetic torpor (ST) is a condition in which the rat, a non-hibernator, can be induced by the central nervous inhibition of thermogenesis. During this condition, Tau protein is hyperphosphorylated (PPtau), in a form representing a key neuropathological hallmark in many neurodegenerative diseases, but recovering euthermia PPtau disappears, eliciting interest for translational goals.
Forty-two male rats were implanted with a microcannula in the Raphe Pallidus, where, after one-week recovery, the GABA-A agonist muscimol was injected for 6h (one injection/h) to induce ST. Brain samples were collected in seven experimental conditions: Control; Nadir of hypothermia; Early Recovery (ER), soon after the return to normothermia; 3h or 6h following ER. Moreover, since an intense sleep bout usually occurs soon after ER, its role in PPtau reversibility was assessed through a 3-h or a 6-h total sleep deprivation (SD) by gentle handling. Tau phosphorylation and the regulation of two functionally involved kinase enzymes (GSK3β and Akt) were assessed by Western Blot.
Results confirmed the reversible brain accumulation of PPtau. This process was speeded up by SD, coherently with a concomitant increase of p9-GSK3β (inactive form) and p(473)-Akt (active GSK3β inhibitor). Data suggest that such a reversible accumulation of PPtau is a finely regulated process, and the effects induced by SD are reasonably driven by the activation of the PI3K/Akt2/mTOR antiapoptotic pathway.

P3.31

Orally administered indicaxanthin is able to modulate human motor cortical excitability and plasticity

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Opuntia Ficus Indica contains Indicaxanthin (IX), an antinflammatory and antioxidant betalaine pigment. This phytochemical is also able to cross the blood brain barrier (BBB) in rats and modulate neuronal activity. Considering these evidence, we aimed at investigating if orally administered IX could affect human brain tissue. 8 healthy and right-handed male subjects were recruited (20-45 years) with no history or clinical signs of neurological diseases, brain trauma or use of drugs acting on neuronal process, as assessed by a clinical neurologist. Non invasive Brain Stimulation and Neuromodulation (NIBS and NIN) instructions were applied in basal condition (T0) and 2 hours after having assumed 400 gr of cactus pear fruits (T1), over one week distance at least. Each subject experienced 30 pseudorandomised stimuli of paired pulse transcranial magnetic stimulation (ppTMS) over the M1: 10 short intracortial inhibition (SICI), 10 intracortical facilitation (ICF) and 10 test stimuli. They were delivered before and after 20 minutes of anodal transcranial Direct Current Stimulation (a-tDCS). IX significantly increased PRE-tDCS TEST ($p<0.0103$) and PRE-tDCS ICF ($p<0.052$), POST-tDCS ICF ($p<0.0001$) and SICI ($p<0.001$) were reduced, PRE-tDCS SICI was unchanged. All considered, IX is able to increase cortical excitability of human motor cortex. Finally, this nutraceutical seems to achieve an excitatory drive on motor cortical plasticity due to the paradoxical effects emerged after tDCS.

P3.32

The speed of optic flow stimuli modulates microsaccades' characteristics

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Microsaccades are linked with extraretinal mechanisms that significantly alter spatial perception before the eye movements onset. We sought to investigate whether the microsaccadic activity was modulated by the speed of radial optic flow stimuli. The experiments were performed in the dark on 20 subjects who stood in front of a screen covering 135 x 107° of visual field. Subjects were instructed to fixate a central fixation point while optic flow stimuli were presented full field, in the foveal and in the peripheral visual field at different dots speed (8, 11, 14, 17, 20 °/s). Fixation in the dark was used as baseline. The eye position was recorded using the EyeLink II (Sr-Research, Canada).
Results showed that the stimulation of the peripheral retina evoked a higher microsaccade rate ($p=0,012$). We also found combined effects of optic flow speed and stimulated retinal region (foveal, peripheral, full field) for microsaccade latency ($p=0,006$). These results show that the optic flow speed modulates the microsaccade activity when presented in specific retinal portions, suggesting that eye movement generation is strictly dependent on the stimulated retinal regions.
In all stimulations, the microsaccades directions were significantly clustered in the upper-left quadrant of the visual field ($p<0,001$). These results agree with a previous study showing that when attention is directed toward the fixation point the microsaccades directions show non-uniform distributions.

P3.33

Effect exerted by the vasoactive intestinal polypeptide on the neurons of the subparaventricular zone

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Introduction: The suprachiasmatic nucleus (SCN) is responsible for generating the circadian rhythmicity in mammals. The ventral SCN neurons express the neuropeptide vasoactive intestinal polypeptide (VIP) which is central for coherency and synchrony of SCN activity. VIP-expressing neurons in the SCN densely project to the ventral subparaventricular zone (vSPZ).
Methods: We studied the effects of VIP on vSPZ neurons in brain slices of mice with combined calcium-imaging and whole-cell patch-clamp recording techniques.
Results: Using GCamp6-based *in vitro* calcium imaging we found that VIP excites 17% of vSPZ neurons and this effect was maintained in the presence of tetrodotoxin (TTX) and synaptic blockers for AMPA/NMDA and $GABA_A$ transmissions suggesting a direct effect of VIP on vSPZ neurons. We confirmed this result with patch-clamp recordings: in the presence of synaptic blockers, VIP produced a membrane depolarization of 29% of vSPZ neurons. In addition, we found that in a small percentage of vSPZ neurons VIP increased the frequency of the glutamatergic excitatory postsynaptic currents, suggesting an additional indirect excitatory mechanism.
Conclusion: We concluded that when VIP is released from the SCN VIP fibers it can activate and excite the vSPZ neurons.

P3.34

Differences in task-difficulty encoding during logical decision-making in prefrontal and premotor cortical activity from non-human primates

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Prefrontal (PFC) and dorsal Premotor Cortices (PMd) have been reported to play a major role in Perceptual Decision-making. However, their contribution in selecting the target item between elements encoded within a memory scheme is still unexplored. Transitive Inference (TI) is one such task, where the subject needs to construct a mental schema comprising a ranked series of items (e.g. A>B>C>D>E>F) and consequently identify the higher ranking item at one of the two spatial locations. The subject's ability to infer the rank of each item suggests a mapping of the items in a mental line, with a partial overlapping of adjacent items, described as Symbolic Distance Effect (SDE). The SDE gives rise to a logical complexity resulting in slower and less accurate decisions for selection between closely located items. To study the neural correlates underpinning these decisions, we recorded the extracellular activity from PFC and PMd of three macaque monkeys while they performed a 6-item TI task. The performance and the reaction times of all the monkeys exhibited a SDE. Easier comparisons elicited a higher neuronal response involving greater number of neurons contributing to spatial selectivity of the target item.
Furthermore, the latencies of this selectivity were found to be lower in both the PFC and the PMd for easier pair comparisons. An inter-area comparison between the two areas revealed that this modulation occurred earlier in PFC than in PMd.

P3.35

Local inhibitory circuits regulate frequency-dependent transmission in the cerebellar molecular layer

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The computational properties of the cerebellar granular layer are not yet fully understood. We combined patch-clamp recordings and detailed computational models to investigate the local microcircuit made of parallel fibers (PF), stellate cells (SC) and Purkinje cells (PC). SCs showed spontaneous firing, adaption and rebounds during depolarization and hyperpolarization, and a marked short-term facilitation (STF) during repetitive PF transmission. The frequency-dependent STF at PF-SC showed a characteristic high-pass profile impacting on the PC output. Simulations showed that, following PFs stimulation, PCs almost linearly increased their input frequency response, but a sufficient number of SC synapses (>= 50) abolished the response of PCs at high frequency (> 50 Hz) without affecting the activity at low frequency (< 50 Hz). When reciprocal inhibitory connections between SCs were activated, the control of SCs over PC discharge was maintained only at very high frequencies. The different frequency-dependence of SC and PC responses to input bursts suggested that SCs could implement an efficient system of filters in the molecular layer regulating PC activation and improving the computational capabilities of the circuit.

P3.36

Functional alterations in a Müller cell line: potential implications in diabetic retinopathy

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Diabetic retinopathy (DR) is a common complication of diabetes mellitus and is the major cause of vision loss in adult-working population. Although DR is traditionally considered a microvascular disease, an increasing body of evidence suggests that neurodegeneration occurs even before the manifestation of any vasculopathy. To better understand the neurodegenerative process occurring in diabetic retina, it is important to explore functional alterations even in non-neuronal cells, such as supportive glial cells. For this purpose, we investigated potential functional changes in Müller cells - the most abundant glial population within the retina - under experimental conditions that mimic those observed in DR patients (i.e. hyperglycaemic, oxidative and pro-inflammatory conditions). By fluorescence microscopy approaches, combined with biochemical, and physiological studies, we investigated on an immortalized Müller cell line (rMC-1) functional parameters, such as activation process, oxidative stress, antioxidant response, calcium homeostasis and mitochondrial membrane potential. Our results clearly demonstrate that hyperglycaemic environment *per se* is well-tolerated by rMC-1 cells, whereas it has a major impact on their antioxidant properties when combined with pro-inflammatory conditions. In conclusion, our results propose a novel *in vitro* model which may prove useful to further investigate potential therapeutic molecules for the early treatment of DR.

P3.37

Antioxidant efficacy of a nature-inspired Nrf2 activator in retinal explants

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Oxidative stress (OS) is the main putative cause of inflammation and neurodegeneration in retinal diseases. For these reasons, in the last few years, to counteract OS-induced retinal neuronal degeneration, new therapeutic strategies have been tuned. In particular, antioxidants of natural origin are receiving increasing attention. Recently, curcumin and diallyl sulfide were combined generating a nature-inspired hybrid dubbed NIH1, which has been described as an activator of transcription nuclear factor erythroid-2-related factor-2 (Nrf2), the master regulator of the antioxidant response. We tested the antioxidant and neuroprotective properties of NIH1 in an *ex vivo* model consisting in retinal explants cultured under OS conditions. In particular, we demonstrated that NIH1 induced Nrf2 activation and the consequent expression of antioxidant enzymes like heme-oxygenase-1 (HO-1) and NADPH quinone oxidoreductase (NQO-1). Moreover, in OS conditions, the strengthening of the antioxidant defence system triggered by NIH1 prevented the increase of retinal intracellular ROS levels, cell death, and gliosis induced by OS up to 6 days of incubation. These results suggest that NIH1 boosts the retinal antioxidant response favoring a neuroprotective action and confirming that counteracting retinal OS using natural compounds could be a good strategy for the prevention or treatment of retinal diseases.

P3.38

New neuroprotective effect of lemon IntegroPectin on neuronal SH-SY5Y cells

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Mediated by reactive oxygen species (ROS), including H_2O_2 and its derivates, oxidative stress alters numerous cellular processes, such as mitochondrial regulation and cell signaling, propagating cellular injury that leads to neuronal death and to incurable neurodegenerative diseases (ND).
In recent years, biological activities and potential therapeutic benefits of natural products and their bioactive compounds in ND have been intensively explored and investigated.
In this study we demonstrated that lemon IntegroPectin, obtained via the innovative method of hydrodynamic cavitation of organic lemon processing waste in water, characterized by a low degree of esterification and enriched in antioxidant bioactive compounds such as flavonoids and terpenes, shows significant neuroprotective, antiapoptotic and antioxidant activity in neuroblastoma SH-SY5Y cells exposed to H_2O_2. Interestingly, the same beneficial effects were not observed when SH-SY5Y cells were treated with the same dose of a commercial citrus pectin, extracted via acidic hydrolysis in hot water, strongly suggesting that method of extraction and the subsequent structure of pectin molecule largely affects its biological function.
Our results, together with the absence of toxicity of this new pectic substance rich in adsorbed flavonoids and terpenes, encourage further studies aimed to investigate its activity in preventing, retarding, or even curing ND.

P3.39

Prefrontal cortex hyperexcitability in the IB2 KO mouse model of autism

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Autism encompasses a spectrum of pervasive developmental disorders (ASD) featured by severe impairment in communication and socialization, by repetitive behaviour and by additional cognitive peculiarities. The IB2 knock-out (KO) mouse has been proposed as a model of ASD, since the human IB2 orthologous gene is virtually co-deleted with the SHANK3 gene in all the Phelan-McDermid syndrome cases and in some ASD cases as well. The prefrontal cortex (PFC) and cerebellum have both been suggested as key circuits in ASD. We have recently shown that the cerebellum undergoes an aberrant increase in the NMDA receptor mediated current with an increment of the excitatory/inhibitory (E/I) ratio, enhanced LTP at mossy fiber-granule cell synapses, and dysfunctions in the center-surround configuration. Since PFC and cerebellum are tightly and bidirectionally interconnected, we sought to identify electrophysiological dysfunctions in the PFC layer 5 (L5) of IB2 KO mice. We performed whole-cell patch-clamp recordings in PFC brain slices of IB2 KO mice that revealed an increased firing frequency, which is associated to slow spike frequency adaptation, in L5 pyramidal cells and voltage-sensitive dye imaging showing an increased E/I balance. The enhanced excitability observed in the PFC layer mirrors that observed in the cerebellar granular layer and confirms the hyperfunctioning of a brain area, i.e., the PFC, that is crucial for ASD also IB2 KO mice.

P3.40

Dendritic processing implements spike-timing dependent plasticity (STDP) in cerebellar Golgi cells

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Timing in the cerebellar circuit is tightly controlled by inhibitory interneurons. Among these, Golgi cells play a primary role in modulating the input to Purkinje cells by receiving inputs on apical and basal dendrites by parallel and mossy fibers, respectively. However, the mechanisms through which these neurons integrate input patterns is still unclear. Recently, theoretical models predicted that spike-timing dependent plasticity (STDP) at mossy fiber-Golgi cells synapse would be a pivotal mechanism of Golgi cells inhibition. Furthermore, a computational model suggested the mechanisms through which Golgi cells dendrites might integrate and process the inputs transmitted by parallel and mossy fibers, implying that dendritic processing and plasticity in Golgi cells would depend on the spike time intervals between these two inputs.
This project investigated Golgi cell STDP using whole-cell patch-clamp recordings in acute mice cerebellar slices. Mossy fibers spikes either anticipated or followed those on parallel fibers with a phase difference of ±75 ms. Mechanistically, NMDA channel unblock at mossy fiber synapses on basal dendrites are the potential coincidence detectors of mossy and parallel fiber activity. This hypothesis is now under experimental testing. In summary, this work shows that dendritic processing is instrumental to STDP and opens new perspectives on the role of Golgi cells in determining learning and plasticity in the cerebellar circuit.

P3.41

Immune cells phenotype modulation in motoneuronal depleted spinal cord targeting sonic hedgehog signaling

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Neurodegenerative diseases are characterized by common mechanisms responsible for the sensing, transduction, and amplification of inflammatory processes that affecting homeostasis contribute to neuronal death. The role of sonic hedgehog (Shh) in neuroinflammatory disorders has been reported. Consistent with this, current studies address clobetasol neuroprotective effects, a pleiotropic molecule also acting as an activator of the canonical Shh signalling pathway. Herein, we aimed at studying behavioural and neuromuscular anti-inflammatory effects of clobetasol in a reductionistic model of motoneuronal depletion induced by a neurotoxic insult. We found that clobetasol reduces behavioural impairment and promotes spinal and muscular compensatory processes. In order to analyse the immune cells population of healthy, lesioned and clobetasol-treated spinal cords, we performed a bioinformatic-assisted analysis of flow cytometry data, characterizing both myeloid cells and B and non-B lymphocytes. Flow cytometry data were analysed by performing a uniform manifold approximation and projection (UMAP), with unsupervised identification of each cluster. We found that increased frequency of inflammatory microglia in motoneuron depleted spinal cord was reverted by clobetasol, also reducing the total amount of B and T NK lymphocytes. Our results encourage clobetasol-mediated Shh targeting as an exploitable strategy to support recovery in denervating and neuroinflammatory disorders.

P3.42

The cerebellar cortex microcircuit functioning investigated by two-photon calcium imaging experiments

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The cerebellar cortex is composed by three layers, in which different neuronal types are geometrically and modularly ordered. This morphological arrangement reverberates on microcircuit activity. The afferent mossy fibers carry information to the innermost granular layer. This is recoded by feed forward and feedback inhibition as well as by different forms of synaptic plasticity and transmitted to the upper molecular and Purkinje cells layers to generate the cortex output. Recent experimental and modelling evidence has revealed that both the granular and molecular layers can act as filters of incoming signals. To decipher the activity of this microcircuit, experimental data acquired from several different cells are critical. Thus, we used a scanless two-photon microscope equipped with a spatial light modulator to record calcium signals from multiple neurons simultaneously, while maintaining single-cell resolution. We investigated the spatial organization of granular layer activity and its modification after long-term synaptic plasticity induction. We performed experiments in which we acquired calcium signals from cells of each layer following an electrical stimulation of the mossy fibers. The simultaneity provided by the system permitted to observe differences in the temporal activation of distinct neuronal types, giving insights into the organization of the local microcircuit.

P3.43

Metabolism and autophagy modulation in heterotopic mouse model of glioblastoma after proton boron capture therapy

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Glioblastoma (GBM) radioresistance is one of the main factors in determining significantly low life expectancy in GBM patients. The urgent need for new approaches has been contributing to the development of innovative radiotherapy regimens aimed at counteracting tumour growth and recurrencies, together with reduced side effects. Proton boron capture therapy (PBCT) takes advantage from the reaction of protons with boron to generate three alpha particles, enhancing effectiveness against tumour cells. Here, in a mouse model of GBM, using photoacoustic-assisted imaging, we first found reduced oxygen levels within the tumor microenvironment overtime, confirming a condition limiting the efficacy of radiotherapy. We then analysed the effects of the combination of protons irradiation and boron phenylalanine treatment in vivo. To highlight the molecular signature of PBCT versus protons irradiated GBM, we performed an RNA-Seq analysis finding differentially modulated pathways involved in response to oxygen levels. Using micro positron emission tomography-assisted scanning in vivo, we analysed GBM ^{18}F-2-deoxy-2-fluoroglucose (FDG) uptake, finding reduced levels in PBCT group as compared to controls; finally, histochemical analysis revealed significantly high levels of autophagy and mitophagy in PBCT *versus* proton irradiated GBM. Our results suggested that a rewiring of metabolic circuits is induced by PBCT, revealing valuable implications for therapeutical strategies.

P3.44

Involvement of AQP4ex In edema tumor associated and in blood bran barrier (BBB) damage in human glioblastoma

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Glioblastoma Multiforme (GBM) is an aggressive and invasive brain tumor, coupled with BBB damage, and consequently edema accumulation. AQP4 localization and aggregation could play an important role in GBM associated edema and in the motility of glioma cells. This study investigates the role of the new AQP4 isoform (AQP4ex) in GBM and in the tumor associated edema. Biopsies from GBM patients were histologically characterized by hematoxylin and eosin staining and analyzed to determine AQP4 isoforms expression levels, supramolecular aggregation and localization. Furthermore, correlation with edema volume was obtained by MRI using edema index (EI) and BBB damage was evaluated by fluorescein levels in biopsies in treated GBM patients. Data showed a strong reduction of AQP4ex levels in tumor, coupled with an alteration of the supramolecular organization and a concomitant delocalization and reduction of AQP4 in the tumor region. AQP4ex levels negatively correlated with EI, indicating an important role of AQP4ex in edema accumulation. Finally, fluorescein levels positively correlated with EI and suggesting a progressive BBB damage occurring during tumor development. Our study suggests a pivotal role of AQP4ex in GBM correlated with tumor infiltration and vasogenic edema.

P3.45

Metabolic rebalancing and neuromuscular plasticity in a mouse model of motoneuronal loss

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Neuronal loss represents the consequence of direct or indirect insults to neurons and the major factor mediating persistent disability. Sonic hedgehog (Shh) is a signalling protein regulating cell fate and differentiation and has been indicated as a critical factor in developing and maintenance of the central nervous system (CNS), thus holding great potential in promoting CNS repair and regeneration. Recent evidences support a neuroprotective and regenerative role of the Shh-signalling activator clobetasol, able to target and to activate the effector of Shh pathway smoothened. Herein we aimed at studying behavioural, neuromuscular and metabolic restorative effects of clobetasol in a mouse model of spinal motoneuronal depletion induced by Cholera toxin-B conjugated to saporin (CTB-Sap). We found that clobetasol ameliorates behavioural impairment and muscle denervation restoring Shh-signalling on resident glial cells and neurons fostering spinal plasticity. We then analysed the effects on muscular trophism and metabolism, finding that clobetasol increases the mean myofiber area and mitochondrial fitness, recovering ATP/ADP ratio and energy charge potential at 6 weeks post-spinal motoneuronal ablation. Our results suggest that clobetasol supports compensatory processes and represents an exploitable approach for denervating and degenerative disorders.

P4.1

Biomechanics of the octopus arm muscular hydrostat

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Muscular hydrostats are organs lacking any rigid structure and relying on a “fixed volume constraint” to generate movement. They possess virtually infinite degrees of freedom and undergo large-scale deformations during motions. Octopus arms are “one of a kind” muscular hydrostats for their impressive complexity of motions built upon the antagonistic action of two main muscle groups.
Despite the growing interest due to the possible implementations into the bio-inspired soft-robotics field, little is known on arm muscles biomechanics and on how it relates to their role in motion.
In this work, we investigated arm muscles tissue organization and biomechanics. We show that, although similar in contractile properties, the two muscles are significantly different in term of activation properties, elastic response and extracellular matrix structure.
Interestingly, these characteristics well support the different structural and functional role of each muscle type during motion. Arm muscles hence reveal specific functional adaptations to arm use in different tasks developed to move and control these incredibly dexterous and redundant appendages.
Taken together, our findings shed new light into *Octopus vulgaris* functional adaptations to control its soft limbs and represent a step forward toward the implementation of bio-inspired soft-robotics limbs.

P4.2

Dual-hemisphere tDCS on parietal operculum does not affect the programming of intra-limb anticipatory postural adjustments

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Evidences show that the postural and focal components within the voluntary motor command are functionally unique (doi: 10.1007/s00221-014-3866-4). However, we found that the SMA processes anticipatory postural adjustments (APAs) separately from the command to focal muscles (10.1016/j.bbr.2015.05.044). So we are still searching for a hierarchically higher area able to process both components. Among these, the parietal operculum (PO) seems to be a good candidate, as it integrates both sensory and motor streams (10.1177/1073858414531657).
We reported that changing the excitability of PO contralateral to the moving segment did not affect intra-limb APAs (10.3389/fphys.2019.01159). In that paper, transcranial direct current stimulation (tDCS) was applied with an active electrode on PO vs. a much larger reference electrode on the opposite forehead. However, literature reports that two active electrodes of opposite polarities, one on each PO (dual-hemisphere tDCS), elicit stronger effects than the "active vs. reference" arrangement.
Thus, APAs stabilizing the arm when the right index-finger is briskly flexed were recorded before, during and after dual-hemisphere tDCS on PO. Ten right-handed subjects were tested for each polarity: anode on the left vs. cathode on the right, and vice versa. Again, tDCS was ineffective on APAs amplitude and timing. Also index-finger kinematic was unchanged.
These results confirm the conclusion that PO does not take part in intra-limb APA central control.

P4.3

Failure of the race model accounting for inhibition in a stop signal selective task of upper and lower limb

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Inhibitory control of movement is essential for interacting with a continuously changing environment. This function has been largely investigated by using the Stop-Signal Task (SST), that requires to execute a movement when a Go signal is presented, and to refrain it as a Stop signal suddenly appears in a minority of trials. While SST and the related theoretical model (race model) have been successfully used to study the behavioural and physiological aspects of motor inhibition for single effectors, it is still poorly investigated how it accounts for inhibition of actions in which more effectors act concurrently. Here we asked if the theoretical model subtending the inhibition of a single effector, it is still valid when inhibition requires to be selective for one of two effectors acting simultaneously. Nine participants were required to rotate the wrist and lift the foot in response to a Go signal and suppress the movement in response to a Stop signal of one selected effector (selective), both effectors (global), or a randomly suppression of either both or one effector (mixed). Our results revealed that the race model correctly accounted for the selective inhibitory performance of both effectors, but failed its prediction in the mixed condition. Present results suggest that when different go processes are running in parallel the interaction with the stop process is less linear.

P4.4

Tongue strength and regular effort saliva swallows measured by piezo-resistive sensors: a test-retest reproducibility study in healthy subjects

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Aim of this study was to establish the reproducibility of tongue strength measurements in healthy subjects during maximum anterior isometric pressure (MAIP) and regular effort saliva swallows (RESS).
Thirty healthy young adults were required to push with the tip of the tongue on a pressure sensor placed on the hard palate. Tongue pressures exerted during MAIP and RESS were recorded. Participants underwent a retest procedure within the same day to verify measurement reproducibility. Intraclass-correlation coefficient (ICC), standard error of measurement (SEM) and minimum detectable change (MDC) were determined. Complete data were obtained from 30 subjects (15 women, 15 men; mean age: 31.4±7.8 years; mean weight: 61.3±9.4 kg). Compared to women, men showed only a trend for generating larger MAIP (p=0.06) and RESS (p=0.07). After normalizing to body weight and height, trends disappeared. At retest, MAIP and RESS were stable and reliable (all ICCs ≥0.93) in both sexes but presented a moderate variability (high SEM and MDC), with MAIP estimates associated to smaller SEM and MDC (SEM 7.4-14.2%; MDC 18.6-20.9%) than RESS (SEM 20.4-38.5%; MDC 52.5-55.6%).
No gender-based differences emerged in the motor tasks tested. Piezo-resistive pressure sensors allow performing reliable measurements of tongue muscle strength. However, measurement variability in tongue performance should be taken into account when appraising the clinical efficacy of therapeutic interventions.

P4.5

Whole-body cryotherapy rebalances the sympathovagal activity both before and after a maximal exercise

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Whole-body cryotherapy (WBC) is a short exposure to air below -100°C known to enhance the physical recovery and to reduce pain and perceived symptoms of muscle soreness (Rose et al, DOI: 10.1055/s-0043-114861). This practice seems to act on metabolism, inflammation, and tissue damage.
Literature mainly studied WBC applied immediately after exercise, while no data are available on the net effect of WBC in the resting periods preceding and following the exercise. Hence, we evaluated the sympathovagal balance both before and after complete recovery from maximal exercise in naïve subjects; then the whole procedure was repeated after WBC (3 min, -100 to -150°C).
ECG was recorded in 11 healthy men during rest, all-out exercise on a cycle ergometer (~30 s), and after recovery. R-R intervals were extracted and HR variability power was measured in the low and high frequency ranges (LF ~0.04-0.15 Hz; HF ~0.15-0.4 Hz) to estimate the sympathetic and the vagal activation, respectively.
As expected, exercise reduced LF and, largely, HF, thus increasing LF/HF ratio up to ~2.5 times. In turn, WBC decreased LF/HF both at rest (from 2.01 to 1.53) and after exercise (from 5.37 to 3.53). Repeated measures ANOVA confirmed this finding with a *frequency* x *exercise* ($p<0.003$) and *frequency* x *WBC* interactions ($p=0.047$), with no three-way interaction.
These results indicate that a single WBC session helps to rebalance the sympathovagal activity with an effect that persists even after a maximal exercise.

P4.6

An intensity-dependent slow component of HR interferes with accurate exercise implementation in post-menopausal women

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A time-dependent dissociation between Heart rate (HR) and metabolism can lead to a misprescription of the intensity ingredient of the exercise dose. **Purpose:** we tested the hypothesis that a slow component of HR (i.e. scHR) occurs in all intensity domains, greater than the slow component of oxygen uptake ($scVO_2$), and we developed an equation to predict it across exercise intensities. **Method:** 18 healthy, post-menopausal women (54 ± 4 years) performed on a cycle-ergometer: *i)* a ramp incremental test for thresholds and VO_{2max} detection; *ii)* 30-min constant-load trials at 40, 50, 60, 70, and 80 $\%VO_{2max}$ for the measurement of the slow component of HR, VO_2, stroke volume (SV) and body temperature (T). scHR and $scVO_2$ were compared by two-way RM-ANOVA (intensity and variable); scHR ($bpm \cdot min^{-2}$) was predicted with a linear model based on exercise intensity relative to the respiratory compensation point (RCP). **Results:** A scHR was present in all domains, greater than $scVO_2$ ($p<0.001$) and significantly correlated with the $scVO_2$ ($r^2=0.46$), scT ($r^2=0.52$), and relative intensity ($r^2=0.66$). A linear equation accurately predicts scHR based on %RCP ($r^2=0.66$, SEE=0.15). **Discussion:** an HR slow component occurred in all exercise domains; its amplitude is twice as large as the VO_2 slow component and is a linear function of %RCP. Such information is essential to grant the desired stimulus is maintained throughout prolonged exercise sessions.

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