74th SIF National Congress

The Italian Society of Physiology

Rome, Italy • 11-13 September 2024

Programme & Abstracts

https://SIF2024.azuleon.org

Programme

Wednesday, 11 September 2024

12:00-19:00

Registration

15:00-17:30

Council of Physiology Professors **[Auditorium]**

15:00-17:30

Young Physiologists meeting **[Aula San Francesco]**

17:30-18:00

Opening Ceremony **[Auditorium]**

18:00-19:00

Opening Lecture **[Auditorium]**

Andrea Volterra *(Lausanne, Switzerland)*

Astrocytes in hippocampal memory and memory impairment

19:00

Welcome cocktail

20:00

Concert "UNE PETITE BARQUE"

viaggio per voce e pianoforte tra musica classica, contaminazioni etniche e jazz **[Auditorium]**

Thursday, 12 September 2024

08:30-09:50

Parallel Symposia

S1 • Active sensing: attention and motor planning [Auditorium]

CHAIR: ROSSELLA BREVEGLIERI (BOLOGNA, ITALY)

Rossella Breveglieri *(Bologna, Italy)*

The medial posterior parietal cortex is a key node of attention for reaching

Paola Binda *(Pisa, Italy)*

Pupillometry gives a signature of implicit learning

Alessandro Benedetto *(Florence, Italy)*

The speed of visual processing changes with eccentricity: a combined EEG and behavioral investigation

Simona Monaco *(Trento, Italy)*

Possible role of attention in the early visual cortex during haptic exploration and motor planning

S2 • Environmental Stressors and Barriers dysfunction: assessing the impact on human health and diseases [Aula A]

CHAIRS: GIULIO SANCINI (MONZA, ITALY) & LEONARDO ERMINI (SIENA, ITALY)

Giulia Terribile *(Monza, Italy)*

The impact of pollution on the neurovascular unit physiology

Cinzia Del Gaudio *(Siena, Italy)*

The effect of environmental stressors on the placenta barrier

Maria Chiara Valerii *(Bologna, Italy)*

Effect of microplastic exposure on intestinal homeostasis

Daniela Gaglio *(Segrate, Italy)*

Micro and nano plastics induce metabolic rewiring and signal transduction alteration in normal human colon cells: a risk factor for human health

S3 • Structure and function of ion channels from experiments and simulations [Aula San Francesco]

CHAIR: LUCA MARAGLIANO (ANCONA, ITALY)

Anna Stary-Weinzinger *(Vienna, Austria)*

Allosteric mechanisms of inward rectifier K^+ channel gating probed by disease mutants and ethosuximide

Arin Marchesi *(Ancona, Italy)*

Structural dynamics of membrane proteins by high-speed atomic force microscopy (HS-AFM)

Simone Furini *(Cesena, Italy)*

Atomistic simulations of C-type inactivation in potassium channels

Luigi Catacuzzeno *(Perugia, Italy)*

Building predictive Markov State Models of ion channel permeation from Molecular Dynamics

09:50-11:00

Parallel Oral Communications

Neurophysiology 1 [Auditorium]

CHAIRS: ANNA MARIA ALOISI (SIENA, ITALY) & MARIA VITTORIA PODDA (ROME, ITALY)

Giuseppe Aceto *(Rome, Italy)*

GPR158 activation decreases Kv7.2/KCNQ/M channel activity and increases intrinsic excitability in medium spiny neurons of the nucleus accumbens

Anna Maria Aloisi *(Siena, Italy)*

Establishment of a cellular model to investigate the role of the pain-insensitive ZFHX2 gene

Roberto Colangeli *(Ancona, Italy)*

"Tune the tone": The endocannabinoid system gates plasticity of tonic GABA inhibition

Laura Maggi *(Rome, Italy)*

Female mice exhibit similar long-term plasticity and microglial function at the ventral and dorsal hippocampal poles

Sara Martini *(Rome, Italy)*

Exploring the role of a chemogenetic LIMK1 analog in dendritic spine dynamics

Stefano Masoli *(Pavia, Italy)*

Filtering properties of a multicompartmental model of the cerebellar basket cell

Francesco Pastore *(Rome, Italy)*

Developing an inducible cAMP response element-binding protein (CREB) to control synaptic plasticity

Maria Vittoria Podda *(Rome, Italy)*

Brain complexity in stroke recovery after bihemispheric transcranial direct current stimulation in mice

Cellular Molecular and Muscle Physiology [Aula A]

CHAIRS: STEFANIA FULLE (CHIETI, ITALY) & ANDREA MARCANTONI (TURIN, ITALY)

Martina Arici *(Milan, Italy)*

YAP1 plays a key role in human embryonic stem cell (hESC)-derived cardiomyocytes maturation

Elena Battirossi *(Sesto Fiorentino, Italy)*

Impact of β- and γ-actin Mutations Leading to Non-Muscle Actinopathies on Cell Cortex mechanics in Neurons

Matteo Moschetta *(Milan, Italy)*

Membrane-targeted photo-actuators for the light-dependent modulation of cell physiology

Luca Palloni *(Milan, Italy)*

AMPK is involved in the long-term regulation of the pacemaker current (I_f) by phosphorylating Ser1157 of HCN4 channels

Giuseppe Petito *(Caserta, Italy)*

Cannabinoid receptor type 1 deficiency triggers a complex adaptative response to improve gastrocnemius muscle function

Anna De Bartolo *(Arcavacata di Rende, Italy)*

Critical implications of the Fc region and FcγRIIA receptor in the side effects of Trastuzumab in human cardiomyocytes

Andrea Marcantoni *(Turin, Italy)*

Defining the effect of calcium released from ryanodine receptors on synaptic function

Physiology of Metabolism [Aula San Francesco]

CHAIRS: LUISA CIGLIANO (NAPLES, ITALY) & SEBASTIANO BANNI (MONSERRATO, ITALY)

Antonella Cano *(Sassari, Italy)*

Extracellular mass to body cell mass ratio (ECM/BCM) as predictor of nutritional, cognitive and functional status in elderly

Gianfranca Carta *(Monserrato, Italy)*

Novel nutritional insights on metabolic flexibility

Luisa Cigliano *(Naples, Italy)*

Modulation of gut-brain axis as a strategy to prevent western diet-induced cognitive dysfunction and stress response in rat hippocampus

Manuela Cipolletti *(Rome, Italy)*

A new link between metabolism and E2 signaling: involvement of PMM2

Anna Patrizia Gena *(Bari, Italy)*

Aquaporin-9 plays a role in the severe metabolic disorder and inflammation in a murine model of lysosomal acid lipase deficiency (LAL-D)

Giulia Querio *(Orbassano, Italy)*

GLUT4 membrane translocation in insulin-stimulated hiPSC-derived cardiomyocytes

Marianna Ranieri *(Bari, Italy)*

p-Coumaric acid prevented the vasopressin-induced water reabsorption through the activation of the Calcium-Sensing Receptor

Concetta Sozio *(Naples, Italy)*

Nutrient excess impairs osteoblast function through increased oxidative stress and impairment of DPP3/Nrf2 pathway

11:00-12:00

Poster Session 1 and Coffee break

12:00-13:00

Plenary Lecture [Auditorium]

Inge Depoortere *(Leuven, Belgium)*

Tick-Tock: significance of circadian clocks in the gut

13:00-14:30

Lunch

14:30-15:50

Parallel Symposia

S4 • New insights into the role of protein post-translational modifications in brain plasticity and neurological diseases [Auditorium]

CHAIRS: SALVATORE FUSCO (ROME, ITALY) & PAUL FRASER (TORONTO, CANADA)

Salvatore Fusco *(Rome, Italy)*

The role of aberrant protein S-palmitoylation in diet- and age-related cognitive decline

Laura Civiero *(Padua, Italy)*

Exploring 14-3-3 interactome perturbations in brain disorders

Claudia Colussi *(Rome, Italy)*

Histone deacetylase 4 regulates the balance of synaptic protein SUMOylation in health and disease

Paul Fraser *(Toronto, Canada)*
Small ubiquitin modifiers as therapies for neurodegenerative diseases

S5 • New and old mechanisms of bidirectional communication between the brain and other organs [Aula A]

CHAIR: GIUSEPPINA D'ALESSANDRO (ROME, ITALY)

Chiara Zurzolo *(Paris, France)*
α-Synuclein-induced autophagy dysfunction in neuronal cells contributes to tunneling nanotube (TNT)-mediated interaction with microglia

Diego Centonze *(Rome, Italy)*
High-fat diet drives glutamatergic synaptic damage by shaping the gut microbiota and T cell dynamics in multiple sclerosis

Stefano Garofalo *(Rome, Italy)*
Platelets-derived serotonin tunes fear memory in mice

Nunzio Iraci *(Catania, Italy)*
Cell-to-cell communication and neuroprotection: insights from astrocyte-derived extracellular vesicles in Parkinson's disease

S6 • Advances in the physiology of breath holding [Aula San Francesco]

CHAIR: ANNA TABONI (BRESCIA, ITALY)

Matteo Paganini *(Padua, Italy)*
Effects of breath-hold diving on gas exchange and the respiratory system

Giovanni Vinetti *(Bolzano, Italy)*
Energy metabolism and energy cost of dynamic apnoea

Antonis Elia *(Stockholm, Sweden)*
Dietary manipulations and their effect on breath-holding

Anna Taboni *(Brescia, Italy)*
Blood pressure control during breath holding

15:50-17:00

Parallel Oral Communications

Cellular and Molecular Physiology 1 [Auditorium]

CHAIRS: MARCO FIOCCHETTI (ROME, ITALY) & MYRIAM CATALANO (ROME, ITALY)

Rosario Amato *(Pisa, Italy)*
Retinal cell resilience to metabolic stress: a putative role of thyroid hormone system

Valentina Brunetti *(Pavia, Italy)*
The mechano-sensitive Piezo1 channel promotes the release of nitric oxide and hydrogen sulphide by triggering global Ca^{2+} signals in the human cerebrovascular endothelial cell line hCMEC/D3 cells

Antonio Cibelli *(Bari, Italy)*
Astrocytes mechanosensitivity to fluid stress is mediated by Piezo1 channel

Marco Fiocchetti *(Rome, Italy)*
Functional role of secreted neuroglobin (NGB) in counteracting neurodegenerative-related cellular stress

Antonio Michelucci *(Perugia, Italy)*
Piezo1 is an essential player in the regulation of cell volume in human glioblastoma cells

Daniele Pensabene *(Pesche, Italy)*
BET proteins inhibition by JQ1 restores redox dyshomeostasis in a cellular model of Parkinson's Disease

Andrea Saponaro *(Milan, Italy)*
Targeting the facilitatory effect of cAMP on HCN2 channel opening promote analgesic actions
Teresa Soda *(Catanzaro, Italy)*
Transient Receptor Potential Ankyrin 1 (TRPA1) mediates hydrogen sulfide-induced Ca^{2+} entry and nitric oxide release in human cerebrovascular endothelium

Neurophysiology 2 [Aula A]

CHAIRS: MARCELLO D'AMELIO (ROME, ITALY) & ROBERTO PIACENTINI (ROME, ITALY)
Maria Conforti *(Pavia, Italy)*
Altered excitatory/inhibitory balance in the prefrontal cortex of the IB2 KO mouse model of autism: from neuronal excitability to cerebellar modulation *in vivo*
Marcello D'Amelio *(Rome, Italy)*
Disentangling dopaminergic deficits along Alzheimer's disease: from experimental models to patients
Antonio De iure *(Rome, Italy)*
Observation of levo-dopa-induced dyskinesias mediated by a wave of depolarization in the striatum
Mattia Lorenzo Di Francesco *(Genoa, Italy)*
Light-dependent inhibition of epileptic-like neuronal hyperexcitability by a photo-activated molecular switch
Paraskevi Krashia *(Rome, Italy)*
Transcranial Alternating Current Stimulation (tACS) at gamma frequency: an up-and-coming tool to slow-down Alzheimer's Disease
Livia La Barbera *(Rome, Italy)*
Midbrain lesion drives hippocampal monoamine drop causing NLRP3-mediated neuroinflammation and Alzheimer's disease-like deficits
Ada Ledonne *(Rome, Italy)*
Targeting ErbB receptors to rescue nigral dopamine neuron hyperactivity and repetitive behaviors in a mouse model of fragile X syndrome
Gilda Loffredo *(Rome, Italy)*
Prefrontal-tDCS activates ventral tegmental area dopamine neurons and ameliorates hippocampal-related cellular, functional and behavioural deficits in a mouse model of Alzheimer's Disease

Neurophysiology & Environmental Physiology [Aula San Francesco]

CHAIRS: DARIN ZERTI (L'AQUILA, ITALY) & ELENA FABBRI (BOLOGNA, ITALY)
Danilo Bondi *(Chieti, Italy)*
Network physiology of hypoxia during rest and isometric exercise
Giulio Sancini *(Monza, Italy)*
Danger in the air: how air pollution can affect brain's physiology
Daniele Borzelli *(Messina, Italy)*
An explicit strategy affects muscle synergy recruitment during adaptation to virtual surgeries
Valentina Ferretti *(Rome, Italy)*
Control of Nesting behavior by PVN-CA2 oxytocin signaling
Jose Fernando Maya Vetencourt *(Pisa, Italy)*
Enriched environment enhances visual responses of the inner retina in early-stage retinitis pigmentosa
Marco Morrone *(Sassari, Italy)*
Anatomo-physiological basis of the cross-education effect: a preliminary study
Umberto Quartetti *(Palermo, Italy)*
The semantics of space: exploring semantic grounding in the right posterior parietal cortex

Darin Zerti *(L'Aquila, Italy)*
Mitochondrial involvement in retinal degeneration and the role of nanoceria in rescuing mitochondrial function

17:00-18:00

Poster Session 2 and Coffee break

18:00-19:00

Herlitzka Lecture **[Auditorium]**

Michael Caplan *(New Haven, CT, USA)*
Renal epithelial cell structure: form, function and malfunction

Friday, 13 September 2024

08:30-09:50

Parallel Symposia

S7 • Computational methods and models for motor control [Auditorium]

CHAIR: ANTONELLA MASELLI (ROME, ITALY)

Mostafa Safaie *(London, United Kingdom)*

Preserved neural dynamics across animals performing similar behaviour

Francesca Sylos Labini *(Rome, Italy)*

Investigating the emergence of modular neuromuscular control of stepping in infants

Emmanuel Guigon *(Paris, France)*

An optimal control framework for the production of limb movements

Marta Russo *(Rome, Italy)*

Which model for interceptive actions?

S8 • Mechanisms of synaptic dysfunction in epilepsies [Aula A]

CHAIRS: GABRIELE RUFFOLO (ROME, ITALY) & MIRIAM SCIACCALUGA (PERUGIA, ITALY)

Mirte Scheper *(Amsterdam, The Netherlands)*

Impaired migration and maturation of SST+ GABAergic interneurons in tuberous sclerosis complex

Gabriele Ruffolo *(Rome, Italy)*

Dysregulation of GABAergic function linked to SST and SST+ interneurons in Tuberous Sclerosis Complex

Miriam Sciaccaluga *(Perugia, Italy)*

Striatal synaptic dysfunctions in a mouse model of Lafora disease

Barbara Bettegazzi *(Milan, Italy)*

Gene therapy strategies to rescue synaptic dysfunction in epilepsy

S9 • Modulation of hippocampal synaptic plasticity and memory [Aula San Francesco]

CHAIR: MARIA ROSARIA TROPEA (CATANIA, ITALY)

Stefano Guglielmo *(Pisa, Italy)*

Episodic memory: from the hippocampal-entorhinal circuit to a distributed network

Maria Rosaria Tropea *(Catania, Italy)*

The cross-talk between Amyloid-β, α7 nicotinic acetylcholine receptors and cyclic nucleotides at the synapse: from physiology to Alzheimer's Disease

Elisa Calcagno *(New York, NY, USA)*

Enhancing memory and synaptic plasticity through histone acetylation modulation: implications for Alzheimer's disease therapy

Domenica Donatella Li Puma *(Rome, Italy)*

A vicious circle among Interleukin-1β, Aβ and Tau affects synaptic function and memory

09:50-11:00

Parallel Oral Communications

Neurophysiology & Physiology of Organs and Systems [Auditorium]

CHAIRS: FABIOLA PACIELLO (ROME, ITALY) & FRANCESCA GRASSI (ROME, ITALY)

Germana Cappellini *(Rome, Italy)*

Development of adaptive locomotion in children with Cerebral Palsy

Edoardo Lecce *(Rome, Italy)*

The greater force exerted by dominant muscles is driven by a higher excitatory input to motoneurons at various contraction intensities

Francesca Montarolo *(Orbassano, Italy)*

Atm deficiency is associated with motor impairment and dysfunction of cerebellar circuitry in mice

Lala Chaimae Naciri *(Milan, Italy)*

Artificial intelligence allows for the automatic identification of taste fungiform papillae

Fabiola Paciello *(Rome, Italy)*

Altered brain networks and auditory dysfunctions in 3×Tg-AD mice underlying early noise-induced memory deficits

Mohammed Zeroual *(Sassari, Italy)*

Sensory afferents from the face modulate cortical sensorimotor integration with topographic specificity and influence face expressions recognition

Mariangela Centrone *(Bari, Italy)*

Tamoxifen impaired the lithium-induced increased cilium length and necroptosis reduction

Sergio Delle Monache *(Rome, Italy)*

Do visual and vestibular perceptual biases mutually compensate during the execution of a self-motion visuo-vestibular task?

Cellular and Molecular Physiology 2 [Aula A]

CHAIRS: VINCENZO MIGLIACCIO (FISCIANO, ITALY) & GIUSEPPINA D'ALESSANDRO (ROME, ITALY)

Amilcare Barca *(Lecce, Italy)*

Ab initio definition of a functional network involving transthyretin, carnosine and copper with (patho)physiological implications in the familial amyloid polyneuropathy (TTR-FAP) rare disease

Giovanna Bastari *(Rome, Italy)*

Function role of Neuroglobin trafficking in mitigating ER-stress response in human neuron derived-cells

Alessio Canovai *(Pisa, Italy)*

Targeting the urokinase-type plasminogen activator receptor system to improve visual function in the rd10 mouse model of retinitis pigmentosa

Angela Ferrulli *(Bari, Italy)*

Downregulation of vasopressin-AQP2 pathway explains the secondary NDI associated with cystinosis: *in vivo* and *in vitro* evidence

Vincenzo Migliaccio *(Fisciano, Italy)*

Protective effects of the extra virgin olive oil phenol compound hydroxytyrosol against palmitate-induced organelle stress and insulin resistance

Agnese Roscioni *(Ancona, Italy)*

Developmental and epileptic encephalopathy-associated mutations cause constitutive opening of the Kv7.2 channel inner pore gate

Simona Scorza *(Bari, Italy)*
Unveiling the role of primary cilia: insights into cAMP signaling dynamics and GPCR functionality using advanced imaging techniques
Grazia Tamma *(Bari, Italy)*
AI-based prospective repurposing investigations for the identification of new promising vasopressin V2 receptor ligands

Neurophysiology 3 **[Aula San Francesco]**

CHAIRS: DAVIDE RAGOZZINO (ROME, ITALY) & CRISTIAN RIPOLI (ROME, ITALY)

Francesca Natale *(Rome, Italy)*
Neural stem cell-derived extracellular vesicles preserve brain plasticity in experimental models of metabolic and neurodegenerative disorders
Davide Ragozzino *(Rome, Italy)*
Time-dependent phenotypical changes of microglia drive alterations in synaptic transmission in acute hippocampal slices
Matteo Spinelli *(Sassari, Italy)*
High fat diet reduces hippocampal dendritic spine density by altering S-palmitoylation of synaptic proteins
Giulia Tomagra *(Turin, Italy)*
Patch-clamp and diamond micro-electrode arrays (µ-D-MEA) recordings to monitor the action of exogenous α-synuclein on dopaminergic neurons' electrical activity and dopamine release
Alessandro Tozzi *(Perugia, Italy)*
Evaluating early synaptic changes in a model of synucleinopathy before overt behavioral alterations
Giulia Urone *(Palermo, Italy)*
New strategies to counteract dopaminergic degeneration: *in vitro* and *in vivo* neurotrophic properties of the first positive allosteric modulator for metabotropic glutamate receptor 3
Valeria Vacanti *(Catania, Italy)*
Blocking dopamine D3 receptors enhances hippocampal synaptic plasticity and memory via post-synaptic mechanisms
Francesca Vacca *(Genoa, Italy)*
A pH-sensitive closed-loop nanomachine to control hyperexcitability at the single neuron level

11:00-12:00

Poster Session 3 and Coffee break

12:00-13:00

Ruzzier Lecture **[Auditorium]**

Britta Engelhardt *(Bern, Switzerland)*
The Brain Barriers maintain CNS Immune Privilege

13:00-14:30

Lunch

14:30-15:30

pH Lecture **[Auditorium]**

David Sulzer *(New York, NY, USA)*
Music math and mind

15:30-16:00

Coffee break

16:00-19:00

SIF General Assembly **[Auditorium]**

YRP 2024 Prizes

Gaia Giuriato *(Verona, Italy)*

Mitochondrial influence on performance fatigability: considering sex variability

Eleonora Pali *(Pavia, Italy)*

Coincidence detection between apical and basal dendrites drives STDP in cerebellar Golgi cells

Serena Canzolino *(Milan, Italy)*

NOD-1 activation increases the spontaneous activity and the I(f) current of murine sinoatrial node cells and alters their response to sympathetic stimulation

20:00

Musei Capitolini private tour & cocktail at Terrazza Caffarelli

Speakers' Abstracts

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

The medial posterior parietal cortex is a key node of attention for reaching

Rossella Breveglieri[1,2], R. Brandolani[1,3], S. Diomedi[1,5], M. Lappe[4], C. Galletti[1], P. Fattori[1,2]
[1]Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna, Italia
[2]Centro Interdipartimentale di Ricerca Industriale Aerospaziale, Università di Bologna, Italia
[3]Università di Camerino, Center for Neuroscience, Camerino, Italia
[4]Dept of Psychology, Otto Creutzfeldt Center for Cognitive and Behavioral Neuroscience, University of Münster, Münster, Germany
[5]Istituto di Scienze e Tecnologie della Cognizione (ISTC), Consiglio Nazionale delle Ricerche (CNR), Padova, Italia

Attention is useful for our manual goal-oriented interactions and plays a key role in motor control. Neuroimaging data show that the medial posterior parietal cortex (mPPC) of monkeys and humans is activated during shifts of covert attention, and electrophysiology experiments have confirmed this evidence at a single-cell level. Also, transcranial magnetic stimulation (TMS) studies with humans further support this involvement. Nevertheless, less clear is the role of this brain region in the interplay between spatial attention and motor control. To look for the neural bases of this interplay, we instructed human volunteers to plan and execute reaching movements while attending to the target, while attending elsewhere, or without constraining attention. During the motor planning, we interfered with the functions of the mPPC with online repetitive TMS. We found that mPPC plays a key role in the spatial association of reach planning and covert attention. We have also found that the level of alertness, measured by pupil size, is a good predictor of the promptness of reach initiation only if we plan a reach to attended targets, and we found that mPPC is causally involved in this coupling. Different from previous understanding, we suggest that mPPC is neither involved in reach planning *per se*, nor in sustained covert attention in absence of a reach plan, but it is specifically involved in maintaining attention to reaching targets.

Pupillometry gives a signature of implicit learning

Paola Binda[1], C. Terzo[2], M. Turi[3], D-C. Burr[2]
[1]University of Pisa, Italy
[2]University of Florence, Italy
[3]Dept of Human and Social Sciences—University of Salento, Lecce, Italy

Far from being a mere reflection of ambient light, pupil diameter has been shown to track the contents of visual perception, the direction of attention and the occurrence of unexpected sensory events. Here we show that pupil-size changes reflect the statistical structure of the sensory stimuli even when they are neither consciously perceived nor within the focus of attention.
We used a frequency-tagging temporal segmentation paradigm (Schwiedrzik and Sudmann, 2020), where sequences of visual images (refreshed at 2 Hz) are displayed either in random order or in pairs, with odd-trial images reliably predicting even-trial images (pairs cycling at 1 Hz). Stimuli were arrays of lines and their numerosity was the only information predicting even from odd trials in the paired-images condition, while the position and orientation of the lines was always randomly resampled on every trial. Participants (N=14) were unaware of the difference between the paired and random conditions. Nevertheless, pupil diameter oscillated at 1 Hz, tracking the statistical structure of the stimulus sequence. The 1-Hz oscillation remained strong even when attention was directed to an irrelevant feature.
In summary, we extracted a pupillary signature of neural prediction in paired images, providing a novel, objective and seamless way to quantify the automatic and implicit structuring of sensory flow into meaningful units.

The speed of visual processing changes with eccentricity: a combined EEG and behavioral investigation

Alessandro Benedetto[1,2,3], S.K. Jenks[1,2], M.V. Mishra[1,2 4], M. Poletti[1,2]
[1]Dept of Brain and Cognitive Sciences, University of Rochester, Rochester, NY, USA
[2]Center for Visual Science, University of Rochester, Rochester, NY, USA
[3]NEUROFARBA Department, University of Florence, Florence, Italy
[4]Dept of Psychology, University of Richmond, Richmond, VA, USA

Despite the vivid experience of a homogeneous vision, our visual system is inherently endowed with highly inhomogeneous structures. While it is established that the temporal characteristics of visual responses vary with eccentricity, the connection between this variation, the speed of visual processing, and its underlying neurophysiological mechanisms remains a topic of debate.
Here, we conducted simultaneous recordings of high-precision gaze positions and EEG activity to investigate how foveal and perifoveal stimulation impact reaction times (RTs) and visual evoked potentials (VEPs).
Volunteers discriminated the position and orientation of a U-shaped figure with the aperture facing either upward or downward. Stimuli were presented briefly either in the foveola (0.33°) or perifovea (6.5°), to the right or left of the fixation point.
When stimuli were equated for sensitivity and cortical area of stimulation, we observed faster RTs in the perifovea condition compared to the foveola (16.8±4 ms). The analysis of VEPs revealed a similar effect for the N1 response latency (11.0±4 ms), a parieto-occipital component associated with discriminative processing. Notably, the eccentricity effect was unaffected by experimental manipulations of spatial attention.
Overall, our findings suggest that visual discrimination speeds vary across eccentricities, with faster processing and shorter latency of early visual responses in the perifovea compared to the foveola.

Possible role of attention in the early visual cortex during haptic exploration and motor planning

Simona Monaco[1], S. Sartin[1], L. Turella[1], D. Crawford[2]
[1]CIMeC - Center for Mind/Brain Sciences, University of Trento, Italy
[2]Centre for Vision Research, York University, Toronto, Ontario

In this talk, I will present a series of research projects that fill a niche in action and perception by investigating their relationship with other forms of cognition, such as motor imagery, and by putting emphasis on the top-down aspects of neural processing. Specifically, I will review fMRI data from three experiments that span three conceptual themes of my ongoing research. First, I will present evidence that haptic exploration of unseen stimulus size can be decoded from the activity patterns within the primary visual cortex and as expected, in the primary somatosensory cortex. Second, I will show that action intention can be decoded as early as in the primary visual cortex even before participants start to move, and that motor preparation differentially modulates the activity pattern in early visual and somatosensory-motor areas. With the third project, I will explain how the neural representations for planning vs. imagining hand movements rely on overlapping but distinct neural substrates in the primary visual cortex, suggesting that these representations cannot be explained merely by visual imagery. These results indicate that action is not only a product of the motor system but rather the unitary output generated by a cascade of neural mechanisms that encompasses the perceptual, motor, and cognitive domains, possibly including attention.

The impact of pollution on the neurovascular unit physiology

Giulia Terribile[1], S. Di Girolamo[1], P. Spaiardi[2], M. Mauri[1], G. Biella[2], S. Sesana[1], F. Re[1,3], G. Sancini[1,3]
[1]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[2]Department of Biology and Biotechnologies, University of Pavia, Pavia, Italy
[3]Nanomedicine Center, University of Milano-Bicocca, Monza, Italy

Assessing the health impact of chronic and sub-chronic exposure to air pollutants (AP) in urban areas is challenging, albeit urgent. In recent years, human epidemiological and animal studies put in evidence how the brain is emerging as an important target for adverse health effects of AP. Indeed, increasing evidence indicates that exposure to AP, combined with individual susceptibility and other possible contributing causes, correlates with the onset or progression of neurodegenerative diseases. Among the different proposed, the two mains are: i) cerebrovascular damage, that can cause altered functions of the blood brain barrier (the so-called hit one, according to “two-hit” vascular hypothesis of Alzheimer disease) and ii) neuroinflammation that itself, or exacerbating the direct damage to the endothelium can lead to neurological impairment.
We thus investigated the direct effect of AP on endothelial cells, astrocytes, microglia and neurons using the standard reference material of diesel exhaust particles (DEP). To evaluate direct and indirect effects, we executed calcium imaging experiments on different brain cells belonging to the neurovascular unit (NVU), which can modulate neurotransmission indirectly and we performed electrophysiological experiments on cortical pyramidal neurons in brain slices.
Our results stated that DEP induces NVU dysfunctions triggering direct and indirect effects potentially linked to the progression of neurodegenerative diseases.

The effect of environmental stressors on the placenta barrier

Cinzia Del Gaudio[1], S. Passaponti[2], S. Nencini[1], L. Cresti[1], R. Romagnoli[1], M. Mandalà[3], A.M. Aloisi[2] , L. Paulesu[1], F. Ietta[1], L. Ermini[1]
[1]Dept Life Sciences, University of Siena, Siena, Italy
[2]Dept Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy
[3]Dept Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy

The appropriate embryo/fetal development is entirely dependent on the intrauterine environment, as well as the proper growth and function of the placenta, the fetal organ that facilitates nutrient and gas exchange between the mother and fetus and acts as a barrier against harmful substances. Environmental stressors like pollutants, chemicals, and physical stress significantly affect placental physiology, leading to severe fetal development and long-term health consequences. Prenatal exposure to endocrine-disrupting chemicals (EDCs) such as bisphenol A (BPA) and diethyl phthalate (DEP), ubiquitous in the environment, poses significant risks to placental integrity and function. These chemicals can either mimic or inhibit hormones like estrogen, progesterone, and thyroid hormones, which regulate transporter proteins for glucose, amino acids, and fatty acid uptake and transfer. *In vivo, ex vivo*, and *in vitro* evidence suggests that BPA maternal dietary exposure alters glucose transporter expression in trophoblast cells. Furthermore, preliminary findings from human chorionic explants exposed to DEP at environmentally relevant concentrations showed that this chemical may impact nutrient transporter protein levels. Research on environmental stressors' effects on the placental barrier is crucial for developing interventions and therapeutic strategies as well as improving maternal and fetal health outcomes in the face of environmental challenges.

Effect of microplastic exposure on intestinal homeostasis

Maria Chiara Valerii
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Microplastics (MPs) are widespread contaminants highly persistent in the environment and present in matrices to which humans are extensively exposed, including food and beverages. Microplastics found in fresh and salt waters derive from cosmetic or medicinal products, but particles referred to as “secondary” are also formed by the progressive fragmentation of plastic waste which, once released into the environment, is degraded by UV radiation or mechanical forces, resulting in the formation of micro-sized particles with diameters < 5 mm. The gastrointestinal system is the most exposed to MPs contamination, with an estimated intake in humans ranging between 81.000 to 123.000 MPs per year. Basing on *in vitro* and *in vivo* experiments, the fate of microplastics after ingestion may be degradation by plastic-degrading bacteria present in the intestine or internalization in intestinal epithelial cells which cause oxidative stress and interferences with cell metabolism. MPs can also cross the intestinal barrier to reach the blood circulation and accumulate in tissues. The impact of microplastics on intestinal homeostasis has been studied *in vivo* with contrasting results, however there is evidence suggesting that ingestion of microplastics can alter gut physiology by affecting the microbial ecology and the epithelial barrier integrity. Here we discuss the effect of MP exposure on microbiota ecology, on metabolome and their possible impact on the intestinal barrier physiology.

Micro and nano plastics induce metabolic rewiring and signal transduction alteration in normal human colon cells: a risk factor for human health

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Metabolomics is a powerful tool for studying how organisms interact with their environment and how these interactions shape diseases related to pollutant exposure. Polystyrene is a thermoplastic polymer widely used in commercial products. Like all plastics, polystyrene can be degraded into microplastic and nanoplastic particles and ingested via food chain contamination. Although the ecological impact due to plastic contamination is well known, there are no studies indicating a carcinogenic potential of polystyrene microplastics (MPs) and nanoplastics (NPs). Here, we evaluated the effects of the MPs and NPs on normal human intestinal CCD-18Co cells. Our results show that internalization of NPs and MPs induces metabolic changes under both acute and chronic exposure by inducing oxidative stress, increasing glycolysis via lactate to sustain energy metabolism and glutamine metabolism to sustain anabolic processes. Along with metabolic rewiring, different molecular pathways related to stress response, such as NRF2-HIF1alpha axis, are altered after plastics exposure We also show that these evidence mirror the effect of the potent carcinogenic agent azoxymethane and HCT15 colon cancer cells, carrying out the typical strategy of cancer cells to optimize nutrients utilization and allowing metabolic adaptation to environmental stress conditions. Taken together our data provide new evidence that chronic NPs and MPs exposure could act as cancer risk factor for human health

Allosteric mechanisms of inward rectifier K^+ channel gating probed by disease mutants and ethosuximide

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G-protein-regulated inward rectifier K^+ channels (GIRKs) underlie the inhibitory effects of major Gi/o coupled neurotransmitters in the heart, glands, and brain and regulate heartbeat, neuronal excitability and plasticity. GIRK channels are involved in pathophysiologies such as drug and alcohol addiction, pain, depression, seizures, and cognitive impairment. Disease-linked mutations revealed an allosteric cross talk between the channel selectivity filter (SF), and the Gβγ binding site. Molecular dynamics (MD) simulations on the G154S mutant provide mechanistic insights into mutation-induced changes, which lead to loss of optimal K^+ coordination and subsequent water-mediated Na^+ flux. Further, aberrant filter dynamics are correlated with dynamic changes at the binding site of the Gβγ dimer, providing information on the constitutive activity of the mutant (Friesacher et al., 2022).
Interestingly, the antiepileptic drug ethosuximide (ETX) suppresses epileptiform activity in a mouse model of GNB1 syndrome, likely through the inhibition of GIRK channels (Colombo et al., 2023). Combining electrophysiology and µs-long MD simulations, we identified an allosteric binding site, close to the physiological activator PIP_2. Remarkably, Gβγ, another physiological activator of GIRKs, increases the potency of ETX block. Our research suggests that ETX is a potent allosteric GIRK blocker and serves as a tool for probing gating-related conformational changes in GIRK.

Structural dynamics of membrane proteins by high-speed atomic force microscopy (HS-AFM)

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HS-AFM has recently emerged as a powerful technique to assess the structure, dynamics, and function of label-free biomolecules at sub-nanometric resolution and video-rate imaging speeds. Here, we showcase the unique potential of HS-AFM to analyze the supramolecular assembly and conformational dynamics of prototypical peripheral and integral membrane proteins under near-native conditions and controlled exposure to environmental stimuli.
Our experiments demonstrate the use of HS-AFM to investigate TMEM16F, a Ca^{2+}-activated ion channel and lipid scramblase, at the single-molecule level. We reveal a range of structurally diverse TMEM16F assemblies, defined by variable inter-subunit dimerization interfaces and protomer orientations, which have escaped prior cryo-EM studies. Additional functional measurements relate this structural multiplicity to lipid and ion permeation processes. We furthermore demonstrate how a HS-AFM setup integrating UV-VIS light sources can be exploited to control and manipulate membrane proteins. Photo-dependent trans-cis isomerization of azobenzene photo-switches is used to induce rapid and reversible aggregation and disassembly of membrane-associated Annexin V proteins into p6- and unusual p3-2D polycrystalline lattices, with potential novel biological activities.
In conclusion, our findings highlight the power of HS-AFM to analyze the structural dynamics of membrane proteins, ranging from large assemblies down to single-molecule protein conformations.

Atomistic simulations of C-type inactivation in potassium channels

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The conductance of several potassium channels decreases over time upon a susteined gating stimulus due to a process known as C-type inactivation. The functional characteristics of C-type inactivation have numerous implications, for instance in channel selectivity and in the regulation of action potential duration, which justifies the interest in the microscopic details of this state transition of potassium channels. The KcsA bacterial potassium channels has long served as a model system to study C-type inactivation. In KcsA, the decrease in conductance is due to a structural rearrengment of the extracellular portion of the pore, where the selectivity filter is located, which closes almost completely during inactivation. Using Molecular Dynamics (MD) simulations, we analyzed the dynamics of the selectivity filter in KcsA and in the voltage-gated hERG channel, together with mutations in the region of the selectivity filter of the two channels that are known to modify the extent and the rate of inactivation. According to these data, the mechanisms of C-type inactivation are different among potassium channels, which might explain the high heterogeinity of this process. This hypothesis of a channel-specific inactivation mechanism is supported by experimental data in other potassium channels, such as Shaker and Trek1.

Building predictive Markov State Models of ion channel permeation from Molecular Dynamics

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Molecular dynamics (MD) simulation of biological processes has always been a very challenging task due to the long timescales of the processes involved and the challenges associated with handling the large amount of output data. Markov State Models (MSMs) have been recently introduced as a powerful tool in this area of research, as they provide a mechanistically comprehensible synthesis of the large amount of MD data and, at the same time, can be used to estimate experimental properties of biological processes. Of the many studies on protein simulation and the MSM-assisted approach, only a few have addressed ion channel permeation. Herein, we propose a method for building an MSM of ion channel permeation that correctly evaluates the current flowing through the channel. This was done by including in the model the definition of a flux matrix carrying information on the charge moving across the channel, suitably built to be used in conjunction with the transition matrix to predict the ion current. The proposed method is also able to drastically reduce the number of states so to obtain an MSM simple enough to be easily understood. Finally, we applied the method to the KcsA channel, obtaining a four-state MSM capable of accurately reproducing the single channel ion current from microseconds MD trajectories.

GPR158 activation decreases Kv7.2/KCNQ/M channel activity and increases intrinsic excitability in medium spiny neurons of the nucleus accumbens

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The Kv7.2/KCNQ/M channels are voltage-gated K+ channels that control numerous aspects of neuronal excitability including setting membrane potential, afterhyperpolarization, and evoked firing. It has been recently discovered that GPR158, a class C orphan G protein-coupled receptor, is a metabotropic glycine receptor. Moreover, it has been shown that its activation increases cAMP levels and PKA activation. Interestingly, both Kv7/KCNQ/M channels and GPR158 are highly expressed in medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a major input structure of the basal ganglia. However, whether glycine modulates the Kv7/M-current through GPR158 activation has not been investigated. Using whole-cell patch-clamp recordings, in voltage-clamp configuration, we found that Kv7/M current amplitudes were significantly reduced in MSNs following glycine application. This effect was absent when glycine was applied in MSNs incubated with PKA or ERK1/2 inhibitors. Moreover, the incubation of NAc slices with glycine increased the levels of phosphorylated ERK1/2. Consistent with the negative modulation of Kv7/M current, glycine-dependent activation of GPR158 increased the firing rate of MSNs. Moreover, selective pharmacological inhibition of the Kv7/M current mimicked and occluded the effects of GPR158 activation on intrinsic excitability. Collectively, our findings suggest that GPR158/PKA/ERK signaling controls MSN excitability via Kv7.2 modulation.

Establishment of a cellular model to investigate the role of the pain-insensitive ZFHX2 gene

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Nociception is the process by which nociceptors recognize strong heat and mechanical stimuli, with their cell bodies in the dorsal root ganglia triggering somatosensory pathways. Exosomes, extracellular vesicles released into the plasma, transmit vital information to target cells. Zinc Finger Homeobox 2 (ZFHX2), a transcriptional regulator expressed in nociceptors, has been detected in urine exosomes and the gene is mutated in six Marsili family members which show a pain-insensitive phenotype. In this study, the F11 cell line was used as an in vitro nociception model, and changes in membrane potential were measured by fluorescent dye assay after exposure or not to an inflammatory cocktail plus exosomes isolated from healthy volunteers, chronic pain patients, and Marsili members. The differentiated F11 cell line showed neuronal excitability markers such as KCNV1 and ZFHX2 protein. Moreover, the differentiated cells showed a higher degree of membrane depolarization than the control cells when exposed to high concentrations of KCl in addition to the inflammatory cocktail. Treatment with exosomes led to a change in membrane potential depending on the group of subjects. These findings suggest that exosomes play a crucial role in modulating nociceptive pathways and could potentially be targeted for pain management therapies. The results show promise for further research on pain sensitivity modulation, opening new avenues for understanding and treating pain at a molecular level.

"Tune the tone": The endocannabinoid system gates plasticity of tonic GABA inhibition

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GABAergic neurotransmission generates two types of inhibitions: the transient activation of synaptic GABA receptors, or phasic inhibition, eliciting inhibitory postsynaptic currents, and the activation of extrasynaptic GABA receptors by “ambient” GABA which causes a persistent, “tonic”, inhibition that finely tunes cellular excitability of target neurons. Change in GABA synaptic efficacy is a crucial mechanism that underlies experience-dependent modifications of brain functions. Several forms of GABA synaptic plasticity require retrograde endocannabinoids (eCB) signaling. Whereas the molecular mechanisms of eCB-dependent presynaptic regulation of GABAergic synaptic strength have been widely reported, little is known about the potential control of eCB signalling on extrasynaptic GABA inhibition. By using whole-cell patch-clamp recordings, we demonstrated that brief depolarization of cortical pyramidal neurons is associated with a transient increase in tonic GABA current that is dependent upon CB1 receptor activity and 2-AG signaling. Moreover, we showed that this depolarization-dependent plasticity of tonic inhibition requires intracellular neurosteroid action since the pharmacological inhibition of steroid production completely abolished this phenomenon. Here we provide evidence of a novel form of eCB-dependent plasticity of tonic inhibition that is likely to have a key role in finely tuning the excitatory-inhibitory balance in the mouse neocortex.

Female mice exhibit similar long-term plasticity and microglial function at the ventral and dorsal hippocampal poles

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The hippocampus is a heterogeneous structure that exhibits functional segregation along its longitudinal axis, with the dorsal part (DH) involved in spatial learning and memory and the ventral part (VH) in emotional responses. Microglia, the CNS's resident macrophages, are known to remodel hippocampal circuits and regulate brain plasticity. Recent studies in male mice have shown that microglia differ between DH and VH, impacting long-term potentiation (LTP) mainly through the CX3CL1-CX3CR1 pathway. Given the significant differences between female and male brains and the critical role of microglia in brain function, we assessed the specific features of the hippocampal poles in female mice. LTP shows similar amplitude in VH and DH and the expression levels of *Cx3cr1* and *Cx3cl1* mRNA do not differ at the two poles. In addition, female mice lacking CX3CR1 on microglia show the same level of LTP. The expression levels of inflammatory markers, known to be involved in plasticity, and phagocytosis markers do not differ in microglia cells isolated from DH or VH. In accordance, microglial ultrastructure and arborization area/perimeter are similar at the two poles. A detailed understanding of the molecular processes underlying microglial sex differences and their potential implications for plasticity in a specific brain region is of major importance in physiological conditions, and it has a potential impact on translational sex- and microglia-specific hippocampal disease.

Exploring the role of a chemogenetic LIMK1 analog in dendritic spine dynamics

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LTP represents the cellular basis for the strengthening of neuronal connections, which play a critical role in learning and memory. Within key cognitive regions such as the hippocampus, LTP relies on the dynamics of dendritic spine morphology and the modulation of synaptic transmission strength. Central to these processes is the role of actin polymerization mediated by the serine/threonine kinase LIM kinase 1 (LIMK1) which regulates the morphology of dendritic spines through the phosphorylation of cofilin, a critical actin-severing protein. We developed a chemogenetic engineered uniRapR-LIMK1, allowing controlling spine enlargement and functional synaptic potentiation and boosting memory in aged mice with reduced cognitive performance. Here, we evaluated the dynamics of uniRapR-LIMK1-mediated potentiated spines using two-photon glutamate uncaging and electrophysiology demonstrating that chemogenetically potentiated spines were neither morphologically nor functionally saturated. Activation of uniRapR-LIMK1 *in vivo,* through administration of AAV-uniRapR-LIMK1, improved cognitive performance and memory encoding in young cognitively intact mice, providing new insights into the mechanisms of learning and potential targets for cognitive enhancement**.**

Filtering properties of a multicompartmental model of the cerebellar basket cell

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Molecular layer interneurons of the cerebellum are classically separated in basket and stellate cells, depending on their localization and on morphological, synaptic, and excitable properties. Basket cells receive excitatory inputs from parallel fibers and then generate a fast inhibitory response on the axosomatic region of Purkinje cells. Here we have experimentally recorded basket cells and measured their intrinsic and synaptic responsiveness. While some properties resembled those of stellate cells, basket cells showed an unusually marked short-term facilitation and operated as low-pass rather than high-pass filters during repetitive parallel fiber stimulation. The analysis of their intrinsic and synaptic activities was simulated over a broad parameter space using multicompartmental computational models, which allowed us to simulate different assets of the parallel fiber - basket cell - Purkinje cell circuit. Following parallel fiber stimulation, Purkinje cell responses were selectively reduced at low-frequency, while leaving the high-frequency regime intact. When reciprocal inhibitory connections between stellate cells were activated, the control of basket cells over Purkinje cell discharge disappeared. These simulations thus predict that basket and stellate cells operate in tandem setting the frequency band of Purkinje cell transmission though the regulation of opposite branches in the frequency/response curve.

Developing an inducible cAMP response element-binding protein (CREB) to control synaptic plasticity

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Learning and memory are fundamental processes of the brain that allow organisms to adapt to their environments by acquiring, storing, and retrieving information. At the cellular level, these processes are primarily supported by synaptic strength and structure changes, a phenomenon known as long-term potentiation (LTP). LTP has an early phase, independent of protein synthesis, and a late phase, dependent on protein synthesis, which involves the activation of transcription factors mainly the cAMP response element binding protein (CREB). However, there is a knowledge gap in spatiotemporally controlling CREB activity in living neurons. Most methods to control CREB activity involve pharmacological agents or genetic manipulation, which can have non-specific effects and are not easily reversible. Here, we present a bioengineered-gated switch approach that uses a new genetically encoded switchable single-chain Tobacco Etch Virus protease (TEV) to control the release of active CREB into the nucleus. Our strategy leads to increased transcription of target genes and induces changes in both the structure and function of synapses. The development of specific and efficient tools to control CREB activity in living neurons would significantly advance our understanding of neuronal plasticity and potentially open up new possibilities for therapeutic interventions for disorders related to learning and memory.

Brain complexity in stroke recovery after bihemispheric transcranial direct current stimulation in mice

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Novel rehabilitation approaches aimed at improving clinical outcomes for stroke survivors are needed. The aim of the current study was to explore the recovery after stroke in mice that underwent a bihemispheric transcranial direct current stimulation (tDCS) treatment, by recording their electric brain activity with local field potential and by measuring behavioural outcomes of Grip Strength test. An innovative parameter that explores the complexity of signals, namely the Entropy, recently adopted to describe brain activity in physiopathological states, was evaluated to analyse local field potential data. Results showed that stroke mice had higher values of Entropy compared to healthy mice, indicating an increase in brain complexity and signal disorder due to the stroke. Additionally, the bihemispheric transcranial direct current stimulation reduced Entropy in both healthy and stroke mice compared to sham stimulated mice, with a greater effect in stroke mice. Moreover, correlation analysis showed a negative correlation between Entropy and Grip Strength values, indicating that higher Entropy values resulted in lower Grip Strength engagement.
Concluding, the current evidence suggests that the Entropy index of brain complexity characterizes stroke pathology and recovery. Together with this, bihemispheric transcranial direct current stimulation can modulate brain rhythms in animal models of stroke, providing potentially new avenues for rehabilitation in humans.

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YAP1 plays a key role in human embryonic stem cell (hESC)-derived cardiomyocytes maturation

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Yes-Associated Protein (YAP1), the downstream effector of Hippo pathway, was shown to be related to pluripotency, mesoderm specification and heart development. Although reports showed evidence for YAP1-dependent heart regeneration and repair following an injury, its role in cardiomyocyte (CM) maturation and function is not fully explored.
We used a genetic model of YAP1 deficient (YAP1-KO) human embryonic stem cell (hESC) to investigate the role of YAP1 in CMs differentiation and function.
First, we characterized effects of YAP1-KO on the transcriptional landscape. In particular, in YAP1-KO CMs late maturation genes were downregulated, while early cardiac commitment markers were upregulated. Moreover, we observed a dysregulation of numerous genes involved in the muscle structure, sarcomere and Z-disc. In fact, YAP1-KO CMs grew slower and did not reach the sarcomere length of WT CMs, suggesting a role of YAP1 in their assembly and maturation.
Patch clamp analysis confirmed the immature/smaller phenotype of YAP1-KO CMs. In fact, they beat slower, they were more depolarized with shorter action potential, justified by reduced I_f, I_{CaL} and I_{Na}. The evaluation of Ca^{2+} transient (Ca_T) parameters in V-clamped Fluo4AM loaded CMs revealed a slower voltage-induced CaT onset and decay, associated to a reduced sarcoplasmic reticulum Ca^{2+} content.
Taken together, these results suggest the relevance of YAP1 in CM sarcomere maturation and differentiation toward a mature phenotype.

Impact of β- and γ-actin Mutations Leading to Non-Muscle Actinopathies on Cell Cortex mechanics in Neurons

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Cellular processes such as migration, division and endocytosis require controlled turnover and remodeling of cytoskeletal actin structures. Non-muscle actinopathies are caused by heterozygous mutations in the genes *ACTB* and *ACTG1*, encoding for cytoskeletal β- and γ-actin, with unclear mechanisms.
We used Dual Laser Optical Tweezers to study the viscoelastic properties of the cell cortex in neural progenitor cells and mature neurons either wild-type or expressing β-(R196H) or γ-(T203M) actin variants causing Baraitser-Winter syndrome.
Ramp-and-hold pull were applied to NPCs and NCs to form tethers, a cell surface protrusion, to determine the mechanical parameters. Tether formation and elongation showed a multiphase force response: during the ramp, an early rise to a peak value is associated with tether formation and followed by a drop to a minimum and a subsequent exponential rise to a steady maximum (F_1); during the hold, an exponential force relaxation to a steady value lower than F_1 . The observed behaviour indicates that the tether behaves as a 2^{nd} order Maxwell viscoelastic element, allowing the mechanical parameters to be estimated as the undamped elastic coefficient (k_0) and the damped elastic and friction coefficients.
In all cell lines, k_0 is unaffected by maturation. In T203M, but not in R196H, k_0 is about 1/5 of that in WT, similar to the decrease observed in cells whit actin polymerization is inhibited by latrunculin. Supported by EJPRD19-033, MUR-2022XJ29R7.

Membrane-targeted photo-actuators for the light-dependent modulation of cell physiology

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We present a family of membrane-targeted azobenzenes (MTs) with push-pull characteristics as a novel method for cell photo-stimulation. These water-soluble molecules naturally integrate into the cell membrane. When exposed to light, they isomerize from trans to cis, altering the local charge distribution and thus triggering a cellular response. Specifically, the photoisomerization of MTs causes distinct and reproducible depolarization. The most promising compound, MTP2, underwent extensive study. Molecular dynamics simulations confirmed the stable integration of MTP2 into the cellular membrane without significantly affecting bilayer thickness. MTP2 was tested across various cell types, including HEK293T cells, primary neurons, and cardiomyocytes, consistently showing steady depolarization. The modulation of membrane potential observed in vitro is attributed to changes in membrane surface charge caused by light-driven alterations in the MT dipole moment within the cell membrane. While not sufficient to trigger action potentials, the rapid light-induced depolarization has potential applications, especially in cardiac electrophysiology. Low-intensity optical stimulation with these modulators could affect cardiac electrical activity, showing promise in destabilizing and terminating cardiac arrhythmias. We foresee the application of MTs in neuroscience, biomedicine, and biophotonics, offering a tool to modulate cell physiology without genetic modifications.

AMPK is involved in the long-term regulation of the pacemaker current (I_f) by phosphorylating Ser1157 of HCN4 channels

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The “funny current” (I_f) is a mixed Na^+/K^+ current expressed in cardiac pacemaker cells, inward and activated on hyperpolarization, that contributes essentially to the generation of spontaneous pacemaker activity and rate regulation. I_f is carried by the hyperpolarization activated cyclic nucleotide gated channel 4 (HCN4), which is predominantly expressed in the native pacemaker cells of the sinoatrial node (SAN). Recent studies show that AMPK is involved in the modulation of mice heart rate due to a decrease in I_f amplitude, but the mechanism underlying this reduction is still unclear. We identified the serine 1157 as the putative AMPK phosphorylation site on HCN4 and confirmed the presence of phosphate groups on this residue by mass spectrometry analysis. Activation of AMPK with AICAR 1 mM (4 hours) reduced the I_{HCN4} amplitude of HEK293T cells transiently transfected with wild-type hHCN4, due to a decrease of current density, i.e. the density of channels expressed on the plasma membrane. Cells transfected with the non-phosphorylatable mutant S1157A were unaffected by AMPK activation, supporting the involvement of this residue.
Preliminary experiments show that AMPK activation reduces I_f of SAN cells from 3-month-old mice, but not from 24-month-old mice. The I_f current density from old mice has the same size as that of AICAR-treated young mice, suggesting that AMPK may be involved in the well-known age-induced bradycardia.

Cannabinoid receptor type 1 deficiency triggers a complex adaptative response to improve gastrocnemius muscle function

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Cannabinoid receptor type 1 (CB1) is located on the skeletal muscle cell membrane, referred to as peripheral CB1 (pCB1). Interestingly, CB1 has also been identified within mitochondria, known as mitochondrial CB1 (mtCB1). This study aims to explore the role of CB1 signaling in the gastrocnemius muscle, assessing physiological processes in both $CB1^{+/+}$ and $CB1^{-/-}$ mice and enhancing our understanding of how CB1 contributes to cellular homeostasis. $CB1^{-/-}$ mice exhibit a substantial miRNA-related alteration in muscle fiber composition, characterized by an enrichment of oxidative fibers. Moreover, the increased in-gel activity of complex I and complex IV indicated that CB1 deficiency affects the functional and structural organization of the mitochondrial respiratory chain. Increased oxidative capacity is associated with elevated oxidative stress and impaired antioxidant defense systems. A dynamic interplay between biogenesis, mitochondrial dynamics, mitophagy, and unfolded protein response (UPR) pathways has been detected in response to CB1 absence. The interconnected mitochondrial network, influenced by increased biogenesis, fusion, and mitochondrial UPR, underlines the dual role of CB1 in regulating both protein quality control and the new mitochondria generation. These findings deepen our knowledge of CB1's impact on muscle physiology and pave the way for further exploration of intricate signaling cascades in the context of CB1 and cellular homeostasis.

Critical implications of the Fc region and FcγRIIA receptor in the side effects of Trastuzumab in human cardiomyocytes

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Fcγ receptors (FcγRs) are involved in several cardiovascular responses. They also participate in the mechanism of action of trastuzumab (TRZ), a monoclonal antibody that exerts anticancer activity by targeting the HER2 receptor, but its blockade in cardiac cells leads to cardiotoxicity, particularly in elderly patients. However, the involvement of FcγRs in TRZ cardiotoxicity is not completely established. Here, we assessed the impact of Fc region in TRZ-induced cardiomyocyte toxicity by evaluating whether a recombinant Fab fragment of TRZ (rFab-HER2) could result in a lower toxicity profile while maintaining TRZ's ability to inhibit HER2 in human cells. Results showed that cells were more susceptible to TRZ-induced cytotoxicity and death than to rFab-HER2. On the other hand, both TRZ and rFab-HER2 negatively modulated HER2 expression and the downstream AKT/ERK pathways. However, unlike TRZ, rFab-HER2 did not affect mitochondrial dynamics, and did not trigger oxidative stress, inflammation, or apoptosis. Moreover, TRZ, but not its Fab fragment, significantly upregulated the expression levels of FCγRIIA, an FcγR expressed in cardiomyocytes and markedly implicated in TRZ-induced antibody-dependent cellular cytotoxicity. Altogether, these results suggest that the Fc region of TRZ can play a critical role in mediating, at least in part, TRZ cardiotoxicity, and identify FcγRIIA as a novel physiological agent whose targeting may help mitigate the cardiomyocyte side effects of TRZ.

Defining the effect of calcium released from ryanodine receptors on synaptic function

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Calcium released from intracellular stores is critical for controlling cellular processes such as secretion, synaptic transmission, and gene expression. Ryanodine receptors (RyRs) are Ca^{2+}-permeable channels found in the endoplasmic reticulum. The current relationship between RyRs and the Ca^{2+}-dependent mechanisms that govern synaptic transmission is unclear. Here, we investigated the role of RyRs-dependent Ca^{2+} release at excitatory synapses by using hippocampal autaptic neurons (hANs) and adrenal chromaffin cells (CCs). We observed that RyRs inhibition through dantrolene administration had no effect on the average amplitude of the evoked Excitatory Post Synaptic current (eEPSC), while significantly reduced the paired-pulse ratio and the amplitude of eEPSCs during high-frequency stimulation. To better understand the involvement of RyRs on the presynaptic machinery, we performed experiments on CCs and focused on the possible interplay between RyRs and non-L type Ca^{2+} channels. We found that dantrolene application increased the mean peak amplitude of non-L type currents by approximately 50% and decreased by ~10% the Ca^{2+}-dependent inactivation of non-L-type Ca^{2+} channels. In summary, our findings indicate that RyRs sustain glutamate release during high-frequency stimulation of hippocampal neurons, while in CCs increase the Ca^{2+}-dependent inactivation of non-L-type Ca^{2+} channels.

Extracellular mass to body cell mass ratio (ECM/BCM) as predictor of nutritional, cognitive and functional status in elderly

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ECM/BCM is a novel parameter of catabolism or extracellular mass expansion. The aim of the study was to assess ECM/BCM as index of nutritional status, functional and cognitive performance in healthy elderly.
Aged subjects (n=286, 66.7±7.613 y.o., female 55,94%) were included. Nutritional status was assessed through Mini Nutritional Assessment (MNA), bioelectrical impedance analysis and 7-day food diary. Cognitive and functional performance were assessed by Montreal Cognitive Assessment (MOCA) and dynamometry, respectively. Plasma total proteins, albumin, and creatinine concentrations were measured. Spearman correlation and linear regression were applied to analyse the data.
ECM/BCM was inversely correlated:1) to MNA (p=0,018), MOCA (p=0,017) and muscle quality index (p<0,0001); 2) to plasma proteins (p=0,007); albumin (p<0,0001) and creatinine (p<0,0001) and 3) to protein intake (p=0,007). Linear regression, weighted by age, showed ECM/BCM as predictor of nutritional (p=0,018), cognitive (p=0,001) and functional (p<0,0001) status.
Our study suggests ECM/BCM as predictor of nutritional, functional and cognitive status in aged population. Nutritional assessment should consider ECM/BCM as marker of nutritional status and global performance in elderly and as proxy to monitor intervention outcomes.

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Novel nutritional insights on metabolic flexibility

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Metabolic flexibility (MF), the body's ability to efficiently use and switch between different energy substrates, such as glucose and lipids, is crucial for adapting to different physiological states. With aging MF decreases, leading to impaired regulation of lipid and glucose metabolism. This impairment can result in hyperglycemia, insulin resistance, ectopic fat accumulation, and is associated with age-related diseases.
We have developed a new Metabolic Flexibility Index (MFI), which quantifies the efficiency of fat utilization as an energy source.
Our analysis shows a positive correlation between the MFI and biomarkers of nuclear receptor activation, particularly PPARα. PPARα promotes the use of fatty acids as energy substrates, suggesting enhanced lipid metabolism efficiency and thus improved MF.
Conversely, a negative correlation was found with the endocannabinoid 2-Arachidonoylglycerol (2-AG), which enhances the motivation to consume food high in carbohydrates and fat. This suggests 2-AG's role in modulating energy balance by promoting energy consumption and limiting MF.
Our findings indicate that MF can be influenced by the interplay between PPARα and the endocannabinoid system. Given the regulatory capabilities of these pathways, especially through dietary interventions, we propose that personalized dietary strategies targeting these pathways could enhance MF. This approach could be an effective measure for mitigating age-related declines in metabolic health.

Modulation of gut-brain axis as a strategy to prevent western diet-induced cognitive dysfunction and stress response in rat hippocampus

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The consumption of western diets (WD), high in fats and sugars, is a crucial contributor to cognitive dysfunction and brain molecular alterations. Therefore, a mandatory challenge is the individuation of strategies able to prevent, via the gut-brain axis, diet-induced impairment of brain physiology. In this study, we explored the impact of a high fat and fructose diet on hippocampus, and the potential efficacy of the probiotic *Limosilactobacillus reuteri* DSM 17938 (*L. reuteri*) in contrasting diet-induced detrimental effects.
Interestingly, impairment in brain memory function, as evaluated by NOR test, the activation of pro-inflammatory signaling pathway, endoplasmic reticulum stress and autophagy was revealed in hippocampus of WD fed rats. All these hippocampal alterations were prevented by *L. reuteri* administration showing for the first time a neuroprotective role of this probiotic.
The WD induced a microbiota reshaping, but *L. reuteri* neither modulated this change, nor the plasma levels of short chain fatty acids, so its efficacy was attributable to the regulation of WD-induced metabolic endotoxemia and systemic inflammation, as decreased levels of LPS and plasma cytokines were found. Further, a protective effect of the probiotic on gut absorption of fructose and its further delivery to brain was evidenced as mechanism involved in brain protection.
The use of probiotics can be beneficial in reversing metabolic syndrome-mediated brain dysfunction and cognitive decline.

A new link between metabolism and E2 signaling: involvement of PMM2

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17β-estradiol (E2), a sex hormone with a broad impact on human physiology, plays a critical role in development, reproduction and serves as a metabolic hormone regulating various cellular metabolic pathways including lipid metabolism. E2 exerts its effects through activation of its receptors, ERα and ERβ, located in the nucleus and at the plasma membrane. E2-ERα complex plays a crucial role in cell proliferation via nuclear and rapid extranuclear signaling. Here, we identify a novel pathway that modulate E2-ERα signaling to cell proliferation. Through screening a library of siRNAs targeting metabolic proteins, we discovered that PMM2 (phosphomannomutase 2), which is involved in the protein glycosylation process, influences E2-ERα signaling. Particularly, PMM2 genetical inhibition reduces E2-dependent ERα transcriptional activity and induces ERα degradation via transcriptional mechanism. Data showed that PMM2 reduction causes a decrease in FOXA1 mRNA and protein levels, which serves as a pioneering factor for ERα. Finally, reducing PMM2 levels re-sensitizes metastatic breast cancer cells to certain endocrine therapy drugs and CDK4/CDK6 inhibitors. These results establish PMM2 involvement in the regulation of E2-ERα signaling to cell proliferation and determine a novel connection between ERα stability and cellular metabolism. Our findings highlight PMM2 as a potential therapeutic target for metastatic ERα-expressing breast tumors, suggesting new avenues for their treatment.

Aquaporin-9 plays a role in the severe metabolic disorder and inflammation in a murine model of lysosomal acid lipase deficiency (LAL-D)

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Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disease due to mutations in the lysosomal acid lipase gene (*LIPA*). Reduced LAL activity leads to a progressive accumulation of cholesterol esters and triglycerides in hepatocytes, adrenal glands, intestine, and macrophage-monocyte system cells throughout the body. LAL-D induces two clinical severity spectra, an infantile form called Wolman disease (WD) and a less aggressive form known as cholesterol ester storage disease (CESD). To date, there is no effective therapy against LAL-D. Here, a transgenic mouse model of CESD (*Lipa*$^{-/-}$ mice) was used to further characterize the LAL-D phenotype and to check the relevance of AQP9, an aquaporin channel highly expressed in liver and leukocytes with pivotal roles in energy balance and inflammation. Compared to healthy control mice, CESD mice revealed early onset of hepatic steatosis already from the 9th day of postnatal life with rapid progression to severe hepatosplenomegaly and microvesicular liver steatosis at day 90 associated with a strong infiltration of neutrophils and Kupffer cells in liver parenchyma. Interestingly, this phenotype was significantly improved after genetic ablation of AQP9 (*Lipa*$^{-/-}$/*Aqp9*$^{-/-}$) indicating an important pathophysiological involvement for AQP9 in the disease and its potential value as drug target since potent and selective AQP9 inhibitors are currently available.

GLUT4 membrane translocation in insulin-stimulated hiPSC-derived cardiomyocytes

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) are a valuable model to deepen the pathophysiological features of the cardiac tissue. The present work focuses on monitoring the insulin response in hiPSC-CM, that after differentiation were incubated in lactate-containing medium.
We performed western blot analysis on total cell lysates from hiPSC-CM and studied the relative expression of two glucose transporters, GLUT4 and GLUT1. The switch towards a higher GLUT4 expression in our model of hiPSC-CM underlined the transition from a fetal to a postnatal phenotype after lactate purification.
We therefore focused on the characterization of the insulin response in our model of lactate-purified hiPSC-CM. Western blots on total cell lysates showed increased phosphorylation of both AKT and AS160 following insulin treatment, highlighting the activation of the intracellular pathway that physiologically induces GLUT4 membrane translocation. To monitor GLUT4 dynamics in response to insulin, western blots of plasma membrane fractions, rather than total lysates, revealed plasma membrane translocation of GLUT4, as occurs in postnatal CM.
These findings suggest that our model of lactate-purified hiPSC-CM shows an insulin response reminiscent of that of a postnatal CM regarding intracellular signaling and GLUT4 translocation to the plasma membrane, thus representing a suitable cellular model in the cardio-metabolic research and personalized medicine field.

p-Coumaric acid prevented the vasopressin-induced water reabsorption through the activation of the Calcium-Sensing Receptor

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The hormone vasopressin (AVP) controls water reabsorption in the renal collecting duct through the regulation of aquaporin-2 (AQP2) expression and trafficking. Several disturbs, including hypertension and inappropriate antidiuretic hormone secretion, are associated with abnormal water balance. Previous studies showed that Olive Leaf Extract (OLE) antagonizes the vasopressin action through stimulation of the Calcium-Sensing Receptor (CaSR). Here, the functional actions of selective polyphenols in OLE were investigated. Acute stimulation of renal collecting duct MCD4 cells with oleuropein, luteolin, and tyrosol at 100, 150, and 200µM elicited a significant increase in CaSR-dependent intracellular calcium release. However, no relevant changes in intracellular calcium levels were measured at lower concentrations. Also, hydroxytyrosol stimulation induced intracellular calcium increases in a CaSR-independent manner. Interestingly, stimulation with p-coumaric acid results in a significant intracellular calcium release at 1nM concentration that was abolished by NPS2143, a selective CaSR inhibitor. In addition, functional studies revealed that p-coumaric acid impaired the vasopressin-induced water reabsorption. These findings suggest that p-coumaric acid antagonizes the vasopressin effects by binding and stimulating the CaSR. Therefore, p-coumaric acid may have beneficial effects in mitigating disorders characterized by abnormal CaSR signaling and affecting renal water reabsorption.

Nutrient excess impairs osteoblast function through increased oxidative stress and impairment of DPP3/Nrf2 pathway

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Nutrient excess (NE), such as glucose (G) and fatty acids, is known to affect osteoblast (OB) mineralizing function by altering cellular metabolism. However, OB cellular response to NE remains to be elucidated. We explored the effect of G and palmitate (PA) excess on OB metabolism by treating OB cultures with G at physiological (5.5 mM) or supra-physiological (HG; 25 mM) concentrations with or without PA (100µM). OB proliferation, clonogenicity, and mineralization were assessed, and mitochondria function was evaluated by fluorescence, qPCR, and oxygen consumption rate (OCR) measurements. Protein analysis was performed on antioxidant molecules. We found that HG and HG+PA treatments negatively affected OB proliferation, clonogenicity, and mineralization vs G alone. We observed reduced mitochondrial size in HG and HG+PA conditions compared to G alone. Increased mitochondrial OCR was detected in HG- and HG+PA-treated OB despite no differences in ATP production compared to G alone. Gene expression analysis revealed increased mitochondrial fatty acid utilization associated with enhanced mitochondrial reactive oxygen species production in HG and HG+PA conditions as compared to G. This was accompanied by an impairment of the antioxidant DPP3/Nrf2 and downstream pathway in HG that was further impaired in presence of PA. Altogether, our data showed that OB functions are affected by nutrient-induced oxidative stress, and this may be relevant in bone metabolism physiology and diseases.

The role of aberrant protein S-palmitoylation in diet- and age-related cognitive decline

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Protein post-translational modifications play a crucial role in the modulation of synaptic function and their alterations are involved in the onset and progression of neurodegenerative disorders. S-palmitoylation is a post-translational modification catalyzed by zinc finger DHHC domain containing (zDHHC) S-acyltransferases that affects both localization and activity of proteins regulating synaptic plasticity and amyloid-β (Aβ) metabolism. We demonstrated that high fat diet (HFD)-induced brain insulin resistance caused LTP and memory impairment due to the accumulation of palmitic acid and increased expression/activation of zDHHC3 leading to hyper-palmitoylation of GluA1 in the hippocampus of mice. Moreover, we found significant increases of both zDHHC7 expression and protein S-palmitoylation in hippocampi of both 3×Tg-AD mice and post-mortem Alzheimer's disease (AD) patients. Finally, both intranasal administration of the palmitoylation inhibitor 2-bromopalmitate and hippocampus-specific knockdown of zDHHC expression abolished the HFD- or AD-related molecular and behavioral changes of brain plasticity and cognition. Our data indicate that aberrant protein S-palmitoylation plays a critical role in hippocampal synaptic plasticity and memory deficits observed in experimental models of metabolic and neurodegenerative disease and suggest zDHHCs as new target for therapeutic interventions against cognitive decline.

Exploring 14-3-3 interactome perturbations in brain disorders

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14-3-3 proteins regulate the activity of numerous cellular proteins (1). This regulation occurs through interactions between 14-3-3 proteins and their binding partners, which are modulated by phosphorylation events occurring both on the client protein and the chaperone itself. In this study, we uncover a novel signaling pathway wherein phosphorylation of 14-3-3 proteins leads to significant changes in their interactome (2). These alterations in protein interactions have been associated with neurological disorders, such as neurovegetative diseases (3).

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Histone deacetylase 4 regulates the balance of synaptic protein SUMOylation in health and disease

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Several studies have emphasized the critical role of HDAC4, an epigenetic regulator, in synapse function, memory, and learning. HDAC4 is normally found in the cytoplasm, but it may translocate to nucleus in response to external stimuli or in a variety of neurological disorders. While HDAC4's nuclear contribution has been related to negative transcription regulation and reduced synaptic function, its cytoplasmic mechanism of action is less understood. Our findings suggest that, in addition to controlling acetylation, HDAC4 may be involved in SUMOylation. Indeed, we discovered that HDAC4 localizes at synapses in WT mice and interacts with synapsin I and several post-synaptic proteins, increasing their SUMO2/3ylation. At the presynaptic level, SUMO2/3ylation of synI improved the interaction with actin, thus enhancing the reserve pool. At the post-synaptic domain, HDAC4 mediated PSD95 and CAMKII SUMO2/3ylation, that increased their synaptic membrane localization and function ameliorating synaptic transmission. Of note, nuclear accumulation of HDAC4 occurred in Alzheimer's disease and in a model of neuroinflammation resulting in reduced synaptic protein SUMO2/3ylation and increased MeCP2-SUMO1ylation, respectively with a negative impact on gene transcription. These data underline the complex mechanism of action of HDAC4 that may rely on distinct and opposite cytoplasmic and nuclear functions depending on the pathophysiological context.

Small ubiquitin modifiers as therapies for neurodegenerative diseases

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Amyloid-β and tau oligomers are key factors in the etiology and synaptotoxicity in Alzheimer's disease (AD) and have been the target for several therapeutic intervention strategies. The goals of this research were to examine the impact of post-translation modification by the small ubiquitin modifier 2 (SUMO2) on AD-related pathology and synaptic loss as well as to develop a SUMO2-based therapeutic for Alzheimer's disease and related disorders. We found that genetically elevated expression of the SUMO2 prevented the cognitive and long-term potentiation (LTP) impairments in a transgenic mouse model of AD amyloid pathology. In addition, systemic administration of a recombinant biologic SUMO2 analogue, termed SBT02, prophylactically halted the progression of AD-associated decline in learning and memory through improved synaptic activity. Remarkably, the SBT02 biologic was equally capable of reversing cognition loss and synaptic impairments in late-stage amyloid pathology relevant to a clinical setting. Mechanistically, SUMO2 and the SBT02 biologic do not alter Aβ processing or clearance, instead they mitigate synaptotoxicity in the presence of high amyloid loads. Enhanced SUMO2 conjugation induced by the SBT02 biologic represents a promising therapeutic strategy to counteract and reverse the toxic effects of amyloid pathology in AD leading to improvements in synaptic plasticity.

α-Synuclein-induced autophagy dysfunction in neuronal cells contributes to tunneling nanotube (TNT)-mediated interaction with microglia

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The neuroimmune system of the brain majorly constitutes microglia, the tissue-resident macrophages of the central nervous system (CNS). These cells play crucial roles in maintaining brain homeostasis. In “resting” state, microglia survey the brain parenchyma to sense any pathological changes, which if detected can bring about “activated” states. Reactive microglia, pathological hallmarks of several neurodegenerative diseases, can be induced by, and negatively affect, neuronal health. Although secretion-mediated communication between neurons and microglia have been well established, contact-mediated communication via Tunneling Nanotubes (TNTs) between these two cell types remain largely unexplored. TNTs facilitate the transfer of aggregated proteins such as Prions, α-Synuclein, and tau, as well as intracellular components like mitochondria between connected cells. Previous reports have demonstrated efficient directional transfer of α-Synuclein (α-Syn) aggregates from neuronal cells to microglia. The reason behind such directionality, however, remains unknown. Using quantitative and live-cell microscopy, we observed differential localization of aggregates on lysosomes of neuronal and microglial cells. Although lysosomal biogenesis increased for both neuronal and microglial cells upon exposure to aggregates, the ability of microglia to target lysosomes for degradation via lysophagy was significantly higher than neuronal cells, both at a basal level and in the presence of aggregates. Further autophagy inhibition in neuronal cells led to heightened aggregate transfer to microglia, also increasing the number of homotypic TNTs between cells. Interestingly, aggregate transfer is also elevated in an inflammatory environment, a neuroimmune phenotype of neurodegenerative diseases. Thereby, we propose a framework of impaired proteostasis and inflammation in mediating intercellular communication between neurons and microglia.

High-fat diet drives glutamatergic synaptic damage by shaping the gut microbiota and T cell dynamics in multiple sclerosis

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High-fat diet (HFD) is one of the environmental factors that induces a systemic inflammatory and aggravates multiple sclerosis (MS). Its impact on the neuro-immune crosstalk remains completely unexplored at synaptic level although inflammatory synaptic dysfunctions are an early hallmark of MS, contributing to a silent disease progression independent of demyelination. Here, we aimed at clarifying HFD effects on inflammatory synaptic damage in MS and its mouse model, the experimental autoimmune encephalomyelitis (EAE). We observed that overweight/obese patients with MS exhibited higher disability and glutamate levels in the CNS. In accordance, HFD strongly worsened neuroinflammation, glutamatergic transmission and the linked clinical manifestations in EAE mice. Unexpectedly, HFD triggered glutamatergic synaptic alterations in CFA mice resembling those observed in EAE. Mechanistically, interleukin-1beta (IL-1β) and tumor necrosis factor (TNF) were identified as pivotal mediators in the process. A multidisciplinary approach revealed that HFD altered blood-brain-barrier permeability and gut microbiota composition, redistributing adaptive immune cells from the periphery into the CNS and leading to glutamatergic synaptic dysfunctions.
Overall, we demonstrated that HFD exacerbates MS and EAE synaptopathy-driven disability by promoting both central and peripheral inflammation, along with gut microbiota dysbiosis. Altogether our findings suggest that reducing dietary fat intake can offer protection against immune-inflammatory synaptic damage and MS progression.

Platelets-derived serotonin tunes fear memory in mice

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Several evidence described the platelet-derived factors as key molecules in the brain-body communication in pathological conditions. Here we identified serotonin as a platelet-derived factor that modulates fear behaviors in mice through the control of inhibitory neurotransmission and plasticity in the hippocampus. Interfering with platelet number or activation reduced hippocampal serotonin and modulated fear learning and memory in mice and this effect was reverted by serotonin replacement by 5-HTP/benserazide. In addition, we unraveled that NK cells participate in this mechanism, regulating IL-13 level in the gut, with effects on serotonin production by enterochromaffin cells and on uptake by platelets. Both NK cells and platelets depletion reduced the activation of hippocampal inhibitory neurons and increased the long-term potentiation of synaptic transmission. The understanding of the role of platelets in the modulation of neuro-immune interactions offers additional tools to the definition of the molecular and cellular elements involved in the growing field of brain-body communication.

Cell-to-cell communication and neuroprotection: insights from astrocyte-derived extracellular vesicles in Parkinson's disease

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Parkinson's disease is characterized by the progressive loss of DAergic neurons in the ventral midbrain (VMB) and their terminals in the striatum (STR). In PD, astrocytes (AS) can have either detrimental or beneficial effects. However, the complex interplay between AS and neurons has not been fully elucidated, yet. Extracellular vesicles (EVs) – membranous nanoparticles containing nucleic acids, proteins etc – efficiently contribute to transfer information between cells.
In our recent work, we demonstrated that AS from the VMB and STR release a population of small-EVs (∼100 nm) in a region-specific manner, with VMB-AS secreting the highest rate of EVs. High-resolution respirometry revealed that both VMB- and STR-AS-EVs recover mitochondrial complex I functionality injured by MPP^+ neurotoxin. Interestingly, only VMB-AS-EVs ameliorate ATP production, supporting a regional specificity in targeting mitochondrial dysfunction.
To investigate the molecular mechanism(s) of neuroprotection exerted by AS-EVs, we modelled how AS-EVs enter target neurons and which are the AS-EV molecular cargoes. The AS-EV content was characterized via small RNA-sequencing and mass spectrometry (MS). Interestingly, MS analysis shows the presence of both cytosolic and mitochondrial proteins, related to neuroprotective pathways. By identifying key molecular players involved in this complex cell-to-cell communication, our findings may pave the way for targeted therapeutic interventions to tackle PD.

Effects of breath-hold diving on gas exchange and the respiratory system

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Humans have mechanisms to survive underwater for a limited amount of time. Increased environmental pressure cause compression of the chest and lung parenchyma; in addition, apnoea and water cause drastic cardiovascular changes. This talk will provide the basis to understand cardiovascular and respiratory adaptations during breath-hold divers (BHDs), with a focus on alveolar-capillary exchanges, blood gas level variations, and the most recent experiments undertaken.

According to Boyle's law and current diving physiology, divers' arterial blood gas (ABG) levels vary proportionally to environmental pressure underwater. As experimentally demonstrated in hyperbaric chambers, the arterial partial pressures of O_2 (PaO_2) in SCUBA divers increase during the descent and progressively normalize when resurfacing. In BHDs instead, PaO_2 rises during the descent - as lungs compress - but progressively falls when resurfacing due to metabolic consumption and redistribution in re-expanding lungs.

Our experiments account for 150 ABGs. After pedaling on the submerged bikes, SCUBA divers developed the predicted hyperoxemia at depth without variations and returned to normal values at the surface.

Instead, in five BHDs out of 14 at -15m, and in 6 out of 20 at -42 m, the predicted hyperoxemia at depth did not develop, probably due to environmental stress exerted on the lungs, resulting in lung edema or atelectasis, which affect gas exchange; various degrees of hypoxemia developed when resurfacing.

Energy metabolism and energy cost of dynamic apnoea

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Introduction. The aim of this study was to evaluate the energy cost per unit distance (C) and the energy system contributions of dynamic apnoea (DA).
Methods. Twelve freedivers (2 female; 5 monofin, 7 bi-fins) performed a 50 m (n=12) and a 100 m (n=6) DA in an Olympic pool. Aerobic energy stores depletion (E_{O2}) was calculated from pre- and post-DA alveolar gas composition and peripheral oxygen saturation, knowing total lung capacity and total haemoglobin mass. Anaerobic lactic energy contribution (E_{La}) was calculated from the increase in capillary blood lactate. Anaerobic alactic energy contribution (E_{PCr}) was calculated by subtracting E_{O2} to the oxygen debt at emersion.
Results. Net (above resting) C was 0.63 ± 0.13 kJ m^{-1} for bi-fins and 0.42 ± 0.08 kJ m^{-1} for mono-fins ($p<0.05$) irrespective of DA distance. Relative E_{O2}, E_{La}, and E_{PCr} were similar between fin types, and were, respectively, 43 ± 11%, 9 ± 4%, and 48 ± 12% in the 50 m trial and 35 ± 10% ($p<0.05$ vs 50 m), 19 ± 4% ($p<0.05$ vs 50 m), and 46 ± 10% in the 100 m trial. The distance traveled per unit of O_2 store depletion was correlated with the DA personal best ($R^2=0.78$).
Conclusions. The C of DA appears to be intermediate between that of shallow water snorkelling and surface swimming. E_{PCr} seems to play an important and previously unrecognised role in the DA energetic balance. Distance swum per unit O2 depleted, but not C, was predictive of performance. These results have implications for DA physiology and training.

Dietary manipulations and their effect on breath-holding

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This study aimed to explore the effect of dietary intake manipulations on breath-holding (apnea) performance in a non-diving cohort. Ten healthy adults attended the laboratory on three separate occasions (48-h apart): after a 14-h fast (F14), 1-h post consumption of a high-calorie, high-carbohydrate (HCHC) or a low-calorie, low-carbohydrate (LCLC) based meal. During each visit, the subjects performed a hyperoxic rebreathing trial and a series of three repeated maximal static apneas. Peripheral oxyhemoglobin saturation (SpO_2), and gas exchange were monitored continuously. At rest, after HCHC, the respiratory exchange ratio (0.87±0.17, p = 0.043) and expired minute volume of carbon dioxide (CO_2; HCHC, 0.35±0.09L/min, p = 0.014) were higher compared with F14 (0.71±0.08; 0.23±0.04L/min) and LCLC (0.72 ± 0.07; 0.25±0.03L/min). A faster CO_2 accumulation was recorded during the HCHC (96 ± 35 s) rebreathing trial (F14, 162±42s, p = 0.001; LCLC, 151±23s, p = 0.002). Longer apneas were reported in F14 compared with HCHC (apneas 1–3, p = 0.046) and LCLC (apneas 2–3, p = 0.006). Moreover, after the third apnea, end-tidal partial pressure of oxygen and nadir SpO_2 were lower in F14 (8.6±2.2kPa, p = 0.028; 77±13%, p = 0.009) compared with HCHC (10.1±1.7kPa; 84±9%). No differences were measured in end-apneic end-tidal partial pressure of CO_2 across diets. Fasting improved apneic performance with apneas being terminated at lower oxygen levels through altering the rate of CO_2 accumulation.

Blood pressure control during breath holding

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During the first few seconds of breath holding (BH) performed in dry conditions, arterial blood pressure (ABP) suddenly falls due to the increase in intrathoracic pressure and cessation of respiratory movements. This ABP fall triggers the arterial baroreflex, which responds with a transient increase in heart rate (HR). An increase in vascular resistance then allows ABP to recover. Afterwards, both ABP and HR remain stable for a few seconds before a final phase appears, characterised by continuous increase of ABP and decrease of HR. This final phase seems to be related to the onset of involuntary respiratory movements and chemoreceptor activation by hypoxia and hypercapnia.
When BH is performed in wet conditions, other reflexes may contribute to the cardiovascular responses. In particular, the trigeminal stimulation by face contact with water is thought to trigger some oxygen preserving mechanisms collectively known as “the diving response”. We investigated the cardiovascular responses to BH in dry (supine, ambient temperature, T=25.0±0.5°C) and wet (prone, water T=29.5±0.3°C) conditions in 16 trained divers (6F). No differences were observed at rest before BH in the main cardiovascular parameters. During BH, HR was lower in wet (60±8bpm) than in dry (73±16bpm, $p<0.05$) BH but no differences were observed in mean ABP or baroreflex response to BH. We concluded that water submersion is sufficient to elicit a greater bradycardic response without influencing the arterial baroreflex.

Retinal cell resilience to metabolic stress: a putative role of thyroid hormone system

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Thyroid hormone (TH) system is known as a key regulator of tissue metabolic status. Metabolic balance is particularly important in the retina, whose high-rated activity renders the organ highly susceptible to metabolic stresses, as in the case of diabetes. Here, we hypothesized that retinal cells may tune the energetic balance and their resilience under metabolic stress condition by modulating TH signaling. In this study, we used the db/db mouse model of type 2 diabetes mellitus to demonstrate that the occurrence of typical hallmarks of retinal diabetic dysmetabolism are paralleled by a local TH reduction, presumably due to the retinal cell upregulation of the inactivating deiodinase 3 (DIO3) and the downregulation of the activating DIO2. Using MIO-M1 retinal cell exposed to high glucose stress, we further established that DIOs alterations is likely triggered by high glucose-induced oxidative stress via Nrf2-HIF-1α pathway and long-termly sustained by the repression of DIO3 post-transcriptional inhibition exerted by miR-133a. Finally, we established that the decrease in TH content under high glucose would preserve MIO-M1 cell integrity by constraining mitochondrial function. Together, these data suggest that retinal cells may reduce TH signaling as a compensatory mechanisms attempting to face high glucose-induced mitochondrial hyperactivity. However, sustained low TH levels may contribute to belated mitochondrial thus increasing retinal cell vulnerability to metabolic stress.

The mechano-sensitive Piezo1 channel promotes the release of nitric oxide and hydrogen sulphide by triggering global Ca^{2+} signals in the human cerebrovascular endothelial cell line hCMEC/D3 cells

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The mechano-sensitive channel Piezo 1 is crucial to mechano-transduction in vascular endothelial cells by driving endothelial cell alignment during the development and shear stress-induced nitric oxide (NO) release, while its role in hydrogen sulphide (H_2S) release is unclear. Herein, we investigated for the first time the expression and role of Piezo1 channels in the human brain capillary endothelial cell line, hCMEC/D3.
The Piezo 1 protein is expressed and mediates robust Ca^{2+} responses to the chemical agonist, Yoda1, which were reduced by the selective Piezo1 blocker, Dooku. Unexpectedly, Yoda1 induced a transient increase in $[Ca^{2+}]_i$ even in the absence of external Ca^{2+}, which was followed by a second rise in $[Ca^{2+}]_i$ by restoring external Ca^{2+} upon agonist washout. Moreover, Yoda1-induced Ca^{2+} release was inhibited by depleting the endoplasmic reticulum (ER) Ca^{2+} pool and blocking the inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$). These findings strongly suggest that Piezo1 also drives ER Ca^{2+} release through the $InsP_3Rs$ followed by Store-Operated Ca^{2+} Entry (SOCE). Finally, Piezo1-mediated Ca^{2+} signals triggered both NO and H_2S release in hCMEC/D3 cells. Taken together, these data suggest that Piezo1 channels could convert a change in the forces experienced by human brain capillary endothelial cells, e.g., during the transit of red blood cells, into Ca^{2+}-dependent signals, i.e., NO and H_2S, which could promote local vasodilation and modulate neuronal activity.

Astrocytes mechanosensitivity to fluid stress is mediated by Piezo1 channel

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Astrocyte endfeet cover brain vasculature, forming the glia limitans that separates perivascular space from brain parenchyma and constitutes a second diffusion barrier in the neurovascular unit. The endfeet also provide a boundary for glymphatic flow of fluid and solutes along and across the astrocyte endfeet into the brain parenchyma, thus influencing fluid distribution, ion balance, and neurotransmitter regulation in the extracellular space. We have examined the sensitivity of primary cultures of mouse astrocytes to shear forces, finding that the Ca^{2+} response (setpoint of 0.1 dyn/cm^2) was modulated by the presence of albumin levels encountered in cerebrospinal fluid under normal and pathological conditions and is abrogated in its absence. Using a pharmacological approach, we have identified that the astrocyte mechanosome involved in the detection of shear stress includes sphingosine-1-phosphate-mediated sensitization of the mechanosensor Piezo1 through the activation of phospholipase C. Fluorescence-quenching water transport assays in astrocyte primary cultures revealed that the rate of cell volume regulation and the extent of cell swelling following hypotonic shock were significantly increased by the Piezo1 agonist and slowed down by the channel-blocking peptide, GsMTx4. Our findings indicated that glymphatic flow through the perivascular space may produce sufficient shear stress to activate astrocytes via Piezo1, thereby potentially modulating parenchymal perfusion.

Functional role of secreted neuroglobin (NGB) in counteracting neurodegenerative-related cellular stress

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Aberrant response to physiological cell stress is part of the mechanisms underlying the development of human diseases, including neuropathologies. Neuroglobin (NGB), a monomeric globin, has gained attention for its role in stress response pathways in neuroprotection. To date, evidence has identified NGB as an inducible protein exerting cell-autonomous neuroprotective functions under various cellular stresses. Notably, recent evidence suggests the extracellular occurrence of NGB.
Here we examined the impact of extracellular NGB on cell response under neurodegenerative-related stresses by using conditioned media (CM) from NGB overexpressing cells to model the extracellular transmission of the protein. Obtained results demonstrated that CM enriched with NGB prevents the early mitochondrial fragmentation and diminishes apoptosis in SH-SY5Y cells exposed to oxidative stress or mitochondrial toxicity. In parallel, we proved the capability of extracellular NGB to mimic the protective effects of endogenous overexpression in reducing cell death under conditions of glucose starvation by counteracting the ER stress response and promoting mitochondrial resilience. Overall, findings reported here demonstrate that the extracellular NGB can amplify the neuroprotective and physiological potential of the globin beyond the cell boundaries, acting as an inducible intercellular compensatory factor capable of triggering protective responses under both stressful and physiological stimuli.

Piezo1 is an essential player in the regulation of cell volume in human glioblastoma cells

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Malignancy of glioblastoma (GBM), the most aggressive human brain tumor, strongly relies on the ability of cells to invade the healthy brain parenchyma. Cell invasion is critically dependent on changes in cell volume and shape driven by transmembrane transport of K^+ and Cl^-. In GBM cells, we found that Ca^{2+}-activated K^+ (K_{Ca}) channels of intermediate (IK) and large (BK) conductance are activated by the hypotonic stimulus as the result of Ca^{2+} influx through mechanosensitive channels (MSCs), mainly Piezo1, and this activation is essential for cell volume regulation. However, two important aspects were not addressed: i), whether Piezo1 is the main MSC mediating Ca^{2+} entry; ii) how BK channels can be activated at the resting voltage of GBM cells. Herein, we generated a stable Piezo1 knock-out GBM cell line using the CRISPR-Cas9 approach and found that K_{Ca} channels activation by the hypotonic stimulus was markedly reduced. By using pharmacological modulators of the store-mediated Ca^{2+} release, we identified a complex Ca^{2+} signaling mechanism operating within specialized compartments made by the close apposition of the plasma membrane (PM) and the endoplasmic reticulum (ER). Specifically, Ca^{2+} influx through Piezo1 channels located in the PM activated the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism through IP_3Rs opening on the ER membrane, bringing the Ca^{2+} concentration within the ER/PM spaces to levels high enough to activate the BK channels even at resting membrane potentials.

BET proteins inhibition by JQ1 restores redox dyshomeostasis in a cellular model of Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. The identified mechanisms of PD neuropathology include mitochondrial deficits, oxidative stress, protein aggregation and autophagy alterations. Unfortunately, the available therapies are exclusively symptomatic, pointing to the need to access new resolutive treatments. In recent years, the activity of BET proteins has emerged as key epigenetic event associated to several neurological disorders. Despite this notion, the role of BET modulation has not yet been investigated in PD. Hence, we aimed at assessing the role of BET proteins inhibitor JQ1 in a rotenone-induced cell model of PD. Our main results show that BET inhibition significantly reduced oxidative stress, promoting the expression and activity of enzymes involved in ROS detoxification. Also, JQ1 boosted mitochondrial mass, hindered α-synuclein aggregation and restored autophagy flux. These effects led to increased cell survival and preservation of neuronal morphology. Overall, these results highlight that BET protein targeting may represent a valuable therapeutic avenue, strongly encouraging to reproduce these findings in more complex *in vivo* models.

Targeting the facilitatory effect of cAMP on HCN2 channel opening promote analgesic actions

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In nociceptors the rate of action potential firing in response to inflammation and nerve injury is controlled via HCN2 channels. HCN channels are activated by voltage and potentiated by the direct binding of cAMP to their cytoplasmic C-terminal region named CNBD (cyclic nucleotide binding domain). To date, the connection between direct cAMP potentiation of HCN2 and nociceptor hyperecitability has only been indirectly demonstrated. In the brain, HCN channels are further regulated by TRIP8b, a cytosolic protein which antagonizes the facilitatory effect of cAMP on channel opening. Given the profound effects of TRIP8b in modulating HCN channel gating, we reckoned that a TRIP8b-based peptide may be employed as a tool for the regulation of HCNs in systems where TRIP8b is not expressed, or is expressed at low levels, including in nociceptors. To this end, we took advantage of our miniaturized version of TRIP8b, $TRIP8b_{nano}$ peptide, which fully recapitulates the affinity and gating effects of full-length TRIP8b. The peptide was employed in isolated nociceptors and in a freely moving rat model of neuropathic pain. After a nerve lesion, rats expressing $TRIP8b_{nano}$ showed no neuropathic pain in response to thermal or mechanical stimuli. The use of $TRIP8b_{nano}$ prove, for the first time, the direct link between chronic pain and the facilitatory effect of cAMP on HCN2 channel opening, thus identifying a new promising therapeutic target for the treatment of chronic pain.

Transient Receptor Potential Ankyrin 1 (TRPA1) mediates hydrogen sulfide-induced Ca^{2+} entry and nitric oxide release in human cerebrovascular endothelium

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The gasotransmitter hydrogen sulfide (H_2S) modulates various brain functions, including neuron excitability, synaptic plasticity, and Ca^{2+} dynamics. Furthermore, H_2S has been shown to stimulate nitric oxide (NO) release from cerebrovascular endothelial cells, thereby regulating NO-dependent endothelial functions, such as angiogenesis, vasorelaxation and cerebral blood flow (CBF). Herein, we provided the first characterization of the mechanisms by which H_2S induces intracellular Ca^{2+} signals and NO release in the human cerebrovascular endothelial cell line, hCMEC/D3. The H_2S donor, sodium hydrosulfide (NaHS), induced a dose-dependent increase in $[Ca^{2+}]_i$ only in the presence of extracellular Ca^{2+}. NaHS-induced extracellular Ca^{2+} entry was mediated by the Ca^{2+}-permeable TRPA1 channel, as shown by pharmacological and genetic manipulation of the TRPA1 protein. Furthermore, NaHS-dependent TRPA1 activation led to NO release that was abolished by buffering the concomitant increase in $[Ca^{2+}]_i$ and inhibiting eNOS. Furthermore, the endothelial agonist, ATP, caused a biphasic increase in $[Ca^{2+}]_i$ that was driven by cystathionine g-lyase (CSE)-dependent H_2S production and by TRPA1 activation. Consistent with this, ATP-induced NO release was strongly reduced either by blocking CSE or by inhibiting TRPA1. These findings provide the first evidence that H_2S stimulates endothelial TRPA1 channels to induce NO production and modulate NO-dependent events at the human neurovascular unit.

Altered excitatory/inhibitory balance in the prefrontal cortex of the IB2 KO mouse model of autism: from neuronal excitability to cerebellar modulation *in vivo*

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Autism spectrum disorder (ASD) are developmental disorders characterized by altered social interactions and repetitive behaviors. Here, we investigated the medial prefrontal cortex (mPFC) alterations and cerebellum-mPFC connectivity in the IB2 KO mouse model of ASD at various levels. *Ex vivo* investigations in the mPFC PreLimbic Region (PrL) of IB2 KO mice revealed: i) disrupted E/I balance in favor of excitation in layer 5 (L5) columns using voltage-sensitive dye imaging; ii) increased excitability and E/I ratio, together with enhanced NMDA receptor-mediated postsynaptic currents in L5 pyramidal neurons using whole-cell patch-clamp recordings. *In vivo* single unit (SU) recordings of putative PrL pyramidal neurons in anesthetized mice were performed to characterize spontaneous activity and responses to electrical stimulation of the cerebellar dentate nucleus (DN). IB2 KO SUs showed lower basal firing frequency and a reduced response to DN stimulation. Interestingly, blocking inhibition had a stronger impact on KO than WT on basal frequency, responses to DN stimulation, and interspike-interval distribution. These results suggest that the PrL neuron hyperexcitability might be compensated *in vivo* by mechanisms boosting the inhibitory modulation. However, additional investigations are needed to explore the balance between primary and compensatory changes in the PrL of IB2 KO mice.

Disentangling dopaminergic deficits along Alzheimer's disease: from experimental models to patients

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The mesocorticolimbic dopaminergic system projects from the Ventral Tegmental Area (VTA) to both cortical and limbic regions and it has been recognized for its central role in motivated behaviors, various types of reward, and in cognitive processes.
In the last few years, we and others proved that physiological functions related to the VTA are progressively deteriorated in validated mouse models of Alzheimer's Disease.
Actually, we demonstrated that the progressive and age-related deterioration of physiological functions in VTA-projecting limbic and cortical regions were strictly dependent on altered dopaminergic signaling.
Furthermore, we proved that the latter disfunctions were timing related to a progressive and selective loss of VTA dopamine neurons occurring in a very early stage of the disease and before Aβ plaque formation.
Our work on AD mice led to many clinical studies on Mild Cognitive Impairment due to AD (MCI) and AD patients that overall describe functional, structural and metabolic changes in the VTA or its projection areas since the MCI stage.
These preclinical and clinical data strongly suggest that damage in the VTA and its targets can be used as an early marker to identify the conversion from healthy state to clinical AD.

Observation of levo-dopa-induced dyskinesias mediated by a wave of depolarization in the striatum

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Spreading depolarization (SD) is a transient self-propagating wave of neuronal and glial depolarization coupled with large membrane ionic changes and a subsequent depression of neuronal activity. SD has been implicated in migraine, stroke, and epilepsy. Conversely, SD in the striatum, a brain structure deeply involved in motor control and in Parkinson's disease (PD) pathophysiology, has been poorly investigated.
We characterized the participation of glutamatergic and dopaminergic transmission in the induction of striatal spreading depolarization by using a novel approach combining optical imaging, measurements of endogenous DA levels, and pharmacological and molecular analyses. Our findings indicate that striatal spreading depolarization requires the concomitant activation of D1-like DA and N-methyl-D-aspartate receptors, and that it is reduced in experimental PD. Chronic L-dopa treatment, which induces dyskinesia in the parkinsonian condition, increases the occurrence and speed of propagation of striatal spreading depolarization. This has a direct impact on one of the signaling pathways downstream from the activation of D1 receptors.
Striatal spreading depolarization might contribute to abnormal basal ganglia activity in the dyskinetic condition and represents a possible therapeutic target.

Light-dependent inhibition of epileptic-like neuronal hyperexcitability by a photo-activated molecular switch

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Photochromic molecules represent a novel strategy for cell-directed modulation of neuronal activity by light stimulation. We characterized in our laboratory a novel azobenzene derivative (Ziapin2), engineered to intercalate into plasma membranes and modulate their electrical capacity in a light-dependent manner. Ziapin2 allows neuronal activity to be modulated in a bidirectional manner: increased membrane capacitance in the dark inhibits firing activity, while the dark-to-light transition generates capacitive currents that evoke action potentials. The properties of such a molecule can thus be exploited to normalize the activity of neuronal networks characterized by an altered level of excitability, under the control of light stimulation. Preliminary data suggest that neuronal hyperexcitability *in vitro*, triggered by the pro-epileptic agent 4-aminopyridine, can be suppressed by the action of Ziapin2, at both the single neuron (patch-clamp) and neural network (Multielectrode Array) levels. We also have insights into how Ziapin2 modulates both excitatory and inhibitory synaptic activity. Further *ex vivo* measurements will be performed on hippocampal slices and *in vivo* on zebrafish larvae to study neuronal electrophysiological activity and neuromotor properties under treatment with 4AP and Ziapin2, thus testing the photochromic molecule in a system characterized by greater complexity and in a translational manner. Funded by “Ricerca Finalizzata 2021” (GR-2021-12374630).

Transcranial Alternating Current Stimulation (tACS) at gamma frequency: an up-and-coming tool to slow-down Alzheimer's Disease

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As the brain performs functions in the awake or sleep states, different brain waves are produced due to rhythmic patterns of neuron activity. Among these oscillations, gamma waves in the 25–140 Hz range have been linked to several cognitive functions, including working memory, learning and attention. In this regard, disorders affecting memory and cognition – such as Alzheimer's Disease (AD) – show reductions in gamma waves. These observations led to recent efforts to restore gamma waves in patients using non-invasive stimulation, with particular emphasis given to transcranial Alternating Current Stimulation (tACS) due to its ability to externally modulate gamma oscillations. Indeed, in mild-to-moderate AD patients, tACS has many positive outcomes such as gamma wave enhancement, cognitive improvement, increased connectivity within the default-mode network, downregulation of pro-inflammatory factors and enhanced Aβ clearance. Yet, works on preclinical AD are scarce and the mechanisms underlying the improvements seen by gamma tACS remain fairly elusive.
Here, we use a validated mouse model of AD, the Tg2576, to first prove deficits in gamma oscillations. In this model we then thoroughly investigate the effects of hippocampal gamma tACS, by focusing on pyramidal neuron function, synaptic plasticity, neuroinflammation, Aβ levels and memory. These data can set the basis for a detailed evaluation of the therapeutic potential of gamma tACS to slow-down AD-related cognitive decline.

Midbrain lesion drives hippocampal monoamine drop causing NLRP3-mediated neuroinflammation and Alzheimer's disease-like deficits

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Midbrain dopaminergic (DA) and serotonergic (5HT) inputs to the hippocampus derive from the ventral tegmental area and interpeduncular nucleus, respectively, and control learning, memory, aversive and stress-coping behaviors. Deficits in these pathways are present since the early phases of Alzheimer's disease (AD), reflecting early mood changes and cognitive decline. Another early event in AD is neuroinflammation, mediated by the activation of NLRP3 inflammasome in glia cells. Recent evidence show that this neuroinflammation is present in midbrain target areas of AD patients. Yet, a direct link between the deficits in midbrain monoaminergic pathways and the insurgence of neuroinflammation in AD is missing.
Here, we test the hypothesis that damage of nuclei producing DA and 5HT induces neuroinflammation in cortical regions of wild-type mice in absence of toxic species. This setting mirrors what happens in preclinical AD, when subcortical changes occur in absence of detectable Aβ. We show that the drop of both DA and 5HT induces hippocampal neuroinflammation, characterized by NLRP3-mediated microglia activation and IL1β release. We also prove that when the midbrain damage is inflicted in pre-plaque stage in Tg2576 mice, a validated AD model, the AD-like phenotype is accelerated with a hyper-inflammatory phenotype associated with increased Aβ load. Overall, our results demonstrate that early monoaminergic midbrain damage induces neuroinflammatory events and worsens AD pathology.

Targeting ErbB receptors to rescue nigral dopamine neuron hyperactivity and repetitive behaviors in a mouse model of fragile X syndrome

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The nigrostriatal dopamine (DA) circuit is key to movement control and creation of habits and sequential behaviors; thus, its dysregulation could promote insurgence of repetitive stereotyped behaviors, core symptoms of autism spectrum disorders (ASD) and fragile X syndrome (FXS), the prevalent genetic cause of intellectual disability and autism. Nevertheless, inspection of substantia nigra pars compacta (SNpc) DA neurons in ASD models has been overlooked and specific evidence of their altered activity in ASD and FXS is absent.
In this study, we provide the first evidence of early hyperactivity of SNpc DA neurons in a FXS mouse model. Moreover, by performing immunofluorescence, western blot experiments, and *in vivo* intracerebral drug injections coupled to behavioral tasks or *ex vivo* electrophysiology, we reveal the molecular substrates of nigral DA neuron dysfunction and repetitive behaviors, that involve the interplay between metabotropic glutamate receptor 1 (mGluR1) and ErbB tyrosine kinases, receptors for the neurotrophic and differentiation factors known as neuregulins. Lastly, we show that systemic administration of an ErbB inhibitor corrects SNpc DA neuron dysfunctions and repetitive behaviors in the FXS model. In conclusion, our data demonstrate that nigral DA neuron hyperactivity is an early signature of FXS, nigral mGluR1 and ErbB play a role in FXS pathology, and inhibiting ErbB is a valuable innovative pharmacological approach to treat ASD and FXS symptoms.

Prefrontal-tDCS activates ventral tegmental area dopamine neurons and ameliorates hippocampal-related cellular, functional and behavioural deficits in a mouse model of Alzheimer's Disease

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A precocious and selective loss of dopaminergic neurons in the Ventral Tegmental Area (VTA) has been reported in Tg2576 mice, a validated model of Alzheimer's Disease (AD). The VTA neurodegeneration in Tg2576 leads to reduced dopamine (DA) in VTA projecting areas including the hippocampus and nucleus accumbens and to deficits of synaptic plasticity, memory and reward processing. Interestingly, DA system deficits have also been associated with a higher risk of conversion from mild cognitive impairment to AD.
Prefrontal cortex Transcranial Direct Current Stimulation (prefrontal-tDCS) is a non-pharmacological and non-invasive brain stimulation method able to activate deep brain structures. Yet, whether tDCS can activate midbrain DA neurons and whether this is sufficient to improve AD deficits still need to be evaluated.
Here, we explored whether a 10-day prefrontal-tDCS in Tg2576 mice (7 or 12 months old) can activate VTA DA neurons and can rescue functional and behavioural deficits.
We showed that tDCS stimulation increases DA hippocampal release, restores synaptic plasticity, and improves declarative memory in Tg2576 mice. Prefrontal-tDCS also ameliorates depressive-like behaviours and hyperlocomotion, two AD-related non-cognitive symptoms. Finally, tDCS-treated mice showed reduced hippocampal neuroinflammation and amyloid plaque burden.
Overall, these data suggest that prefrontal-tDCS could be a promising tool to recover dopaminergic dysfunction underlie very early stage of AD.

Network physiology of hypoxia during rest and isometric exercise

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This cross-over study aimed to capture the integrated response of hypoxia and exercise by comparing three normobaric conditions: control, simulated altitude of 2,500 m (FiO_2≈15.1%) and 3,500 m (FiO_2≈13.5%). The 12 participants (6 m and 6 f; 22.25±2.42 y; 23.01±3.24 kg/m^2) spent ≈ 30 minutes in the tent of Everest Summit II altitude system (Hypoxico, USA), whose last 3 minutes consisted of a series of 9 unilateral isometric maximal contractions of quadriceps. The K5 Wearable Metabolic System (COSMED, Italy) in B×B mode was used to register cardio-respiratory variables. In-degree, out-degree, and transfer entropy (TE) were computed as network variables. The weighted Jaccard Similarity Index was used to evaluate network similarities. The increase of $\dot{V}O_2$ in exercise over rest was slightly more prominent during hypoxia ($p=0.054$, $\eta^2_p=0.232$). Networks were more similar across conditions during resting than exercise. Rest-exercise networks were less similar to each other during a simulated altitude of ≈2,500 m ($p=0.008$, $\eta^2_p=0.353$). Neither TE during rest and exercise nor the SpO_2/FiO_2 ratio significantly predicted the occurrence of symptoms. Unexpectedly, low-grade hypoxia promoted changes in physiological connectivity more than mild-grade hypoxia, where most of the connections converge on putative hidden nodes that we propose are dependent on oxygen delivery. Exercise and environmental physiology benefit from the implementation of network approaches.

Danger in the air: how air pollution can affect brain’s physiology

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Epidemiological studies put in evidence how the brain is emerging as an important target for adverse health effects of airborne pollutants (AP). We thus investigated the direct effect of AP on hCMEC/D3, astrocytes, microglia (MG) and neurons using the standard reference material of diesel exhaust particles (DEP). To study the DEP impact on purinergic signalling, we compared untreated primary MG with those incubated with DEP. Our data showed that DEP-activated MG generated much smaller ATP-induced Ca^{2+} waves, revealing a significant suppression of the receptor-evoked Ca^{2+} signals. The same results have been exactly replicated by hCMEC/D3. We then performed electrophysiological recordings in brain slices by means of Whole-Cell-Patch-Clamp on cortical pyramidal neurons. DEP induced a decrease of sEPSCs/sIPSCs frequency, indicating a pre-synaptic reduction of both excitatory and inhibitory neurotransmitter release. The overall outlined results stated that DEP induces Neuro-Vascular Unit dysfunctions. Finally, in light of our previous results related to the activities of multifunctional liposomes (mApoE-PA-LIP) we demonstrated that mApoE-PA-LIP rescued DEP impaired synaptic functions. Our results clearly evidenced that DEP produces direct and indirect modifications on physiological mechanisms of the synaptic activity effectively linked to the onset and progression of neurodegenerative diseases, for which mApoE-PA-LIP could be promoted as innovative therapeutic tools.

An explicit strategy affects muscle synergy recruitment during adaptation to virtual surgeries

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When exposed to a perturbed environment or when acquiring a new motor skill, both explicit and implicit learning processes are involved. Motor learning often also requires changing muscle activation patterns, which may be generated by recombining existing muscle synergies or learning new synergies. This study examines how explicit cues impact a motor learning in a modular control architecture.
Thirty-six participants practiced 'virtual surgeries', perturbations in the pulling directions of muscles during an isometric force-reaching task in virtual reality. Participants were divided into four groups: those practicing compatible surgeries (requiring recombination of original synergies) or incompatible surgeries (requiring new muscle synergies), and each group received or did not received explicit cues on which muscle was more effective to overcome the surgery.
The presence of the cue increased the number of successful trials, but only when slight muscle alterations were required. These results suggest that an explicit strategy promotes the recombination of existing synergies, rather than a modification of their structure.

Control of Nesting behavior by PVN-CA2 oxytocin signaling

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Nest-building is an innate, extremely conserved behavior serving vital purposes such as protection from predators, regulation of body temperature and sleep.
Nest building ability is strongly modulated by pregnancy: changes in hormones levels promote preparatory activity to ensure the healthy development of the offspring. Perturbations during this sensitive time window have the potential to affect multiple parallel developing systems, and impact the social, cognitive and emotional development of offspring, increasing the incidence of neurodevelopmental disorders. However, to date, the brain mechanisms underlying nesting behaviour remain elusive.
Oxytocin (OXT) is a nonapeptide synthesized in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus mediating a broad range of social behaviours and facilitating adaptation through changing environments.
We found that chemogenetic inhibition of OXT release form the PVN to the CA2 of the hippocampus selectively impairs nesting behaviour in female pregnant mice, producing no effects on maternal care or other social or cognitive functions. Remarkably, in male mice, the same treatment did not affect nesting behaviour, but reduced sexual interaction.
Together, our results identify a sex-specific substrate of the oxytocin brain pathway, paving the way for further studies on the impact of the maternal environment on offspring development.

Enriched environment enhances visual responses of the inner retina in early-stage retinitis pigmentosa

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Sensory experience plays a key role in development and function of the nervous system. We evaluated whether environmental enrichment (EE), a non-invasive condition of enhanced sensory-motor stimulation, promotes the recovery of visual functions in a murine model of retinitis pigmentosa (RCS rats). Using electroretinograms (ERGs), we found that exposing adult blind animals to one month of EE rescues visual responses of the inner retina. Both pattern-ERGs and oscillatory potentials were significantly enhanced after EE as compared to controls; this occurring in parallel to a marked loss of flash-ERGs. Interestingly, multifocal ERG signals increased significantly in RCS rats following enrichment thus showing enhanced local responses, not global, of the external retina to light stimulation. This was accompanied by a behavioral enhancement of light sensitivity and spatial resolution, assessed using the light-dark box test and the optokinetic response. The electrophysiological and behavioral recovery of visual functions paralleled a significant increase in the density and length of perfused retinal capillaries, evaluated by *in vivo* fluorescence microscopy. RT-qPCR revealed an enhancement of retinal VEGF expression levels. Histological and immunohistochemical analysis confirmed all electrophysiological data. Our findings support the application of an enriched environment, alone or in combination with other therapeutic approaches, to rescue visual functions in degenerative blindness.

Anatomo-physiological basis of the cross-education effect: a preliminary study

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Corpus callosum (CC) projections between key cortical motor areas is the most credited mechanism mediating the cross-education (CE), i.e., the physiological phenomenon by which unilateral muscle training increases strength and skills in the contralateral, untrained homologous muscles The relationship between CC subregions connecting these areas and CE magnitude is still unknown. By this pilot trial, we aimed at testing the relationship between the various CC subregions and CE magnitude, using multiple sclerosis (MS) as a model for CC lesions.
Nine subjects with MS, displaying asymmetry in the ankle dorsiflexion muscles (DF) and CC lesions, sustained a 6-week, 3-days per week, maximal isokinetic training of the stronger DF. Muscle strength was assessed before and after training by measuring peak torque (PT) at 10°/s. Lesions' volume in each CC subregion was quantified on MRI scans by semi-automated segmentation techniques.
Our sample showed a significant increase in PT in the untrained limb (+34.9%, $p<0.01$). Spearman correlations revealed a significant association between CE and anterior CC midbody lesion volumes ($\rho=-0.67$, $p=0.04$). No other significant associations were found.
Data confirmed CE occurrence despite CC damage. However, the CC lesional burden negatively impacted the magnitude of CE, in particular in the anterior midbody part of the CC, confirming the hypothesis of CC involvement in strength transfer from the trained to the untrained side.

The semantics of space: exploring semantic grounding in the right posterior parietal cortex

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This study examines the evolving perspective on semantic processing, which has shifted from the traditional view of an isolated semantic memory system to one that recognizes the involvement of distributed neural networks. Recent evidence has supported the notion that semantic processing engages both modality-specific and multimodal regions, with the latter serving as integrative "semantic hubs." In this context, this study focuses on the posterior parietal cortices and their role in processing space-related semantics. We utilized a low-frequency repetitive TMS protocol targeting the posterior parietal cortices across two tasks to investigate this role. The first task required participants to read aloud words from various semantic categories, including those related to space, while the second task entails responding aloud in a dichotomous manner to a question that either involves spatial relations or does not. Despite the first task results not yielding significant findings related to our hypothesis, the outcomes of the second task, and particularly the increased reaction times when spatial terms were used within sentences to establish spatial relationships, shed light on the multifaceted functions of the right posterior parietal cortex, highlighting its potential role in the semantic processing of space-related words. This finding provides insight into the distributed nature of semantic networks and the specialized contributions of modality-specific areas.

Mitochondrial involvement in retinal degeneration and the role of nanoceria in rescuing mitochondrial function

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Mitochondrial dysfunctions have been implicated in the pathophysiology of several age-related diseases including Age-related Macular Degeneration (AMD), the leading cause of vision blindness in the elderly affecting primarily the retinal pigment epithelium (RPE) and photoreceptors. To date, there are no effective therapies to counteract the AMD progression towards the most severe stages characterized by RPE dysfunction. Several studies have shown that a single intravitreal injection of Cerium Oxide nanoparticles (CeO_2-NPs, nanoceria particles), antioxidant agents with auto-regenerative properties, were able to preserve physiological conditions in an animal model of AMD. To improve the knowledge on the mechanisms of action of nanoparticles we performed Transmission Electron Microscopy and molecular analysis, paying particular attention to mitochondrial changes in morphology and function. Our results demonstrated, for the first time, the ultrastructural CeO_2-NPs localization mainly in the RPE and the subretinal space. Furthermore, we demonstrated that nanoceria particles were able to maintain the mitochondrial structural integrity and dynamics, as showed by FIS-1 (fission) and OPA-1 (fusion) modulation. Taken together, our study further corroborated that CeO_2-NPs represent an eligible candidate to counteract oxidative e stress and, therefore, a valid therapeutic agent in retinal neurodegenerative processes.

Preserved neural dynamics across animals performing similar behaviour

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Animals of the same species exhibit similar behaviours that are adapted to their body and environment. These behaviours are shaped at the species level by selection pressures over evolutionary timescales. Yet, it remains unclear how these common behavioural adaptations emerge from the idiosyncratic neural circuitry of each individual. The overall organisation of neural circuits is preserved across individuals because of their common evolutionarily specified developmental programme. Such organisation at the circuit level may constrain neural activity, leading to low-dimensional latent dynamics across the neural population. We suggest that the shared circuit-level constraints within a species would lead to suitably preserved latent dynamics across individuals. We analysed neural activity from monkey and mouse motor cortex to demonstrate that neural dynamics in individuals from the same species are surprisingly preserved when they perform similar behaviour. Neural population dynamics were also preserved when animals planned future movements without overt behaviour. Furthermore, we found that preserved neural dynamics extend beyond cortical regions to the dorsal striatum. Finally, we used neural network models to demonstrate that behavioural similarity is necessary but not sufficient for this preservation. We posit that these emergent dynamics result from evolutionary constraints on brain development and thus reflect fundamental properties of the neural basis of behaviour.

Investigating the emergence of modular neuromuscular control of stepping in infants

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When does modular control of locomotion emerge during human development? We studied this issue in the context of locomotor development. One view is that modularity is not innate, being learnt over several months of experience. Alternatively, the basic motor modules are present at birth, but are subsequently reconfigured due to changing brain-body-environment interactions. Using both simulated and experimental muscle activity data from stepping neonates, infants, and adults, we dissected the influence of noise, and identified modular structures in all individuals. We recorded the EMG activity of 8 bilateral lower limb muscles during stepping/walking in 11 neonates, 53 infants, and 15 adults. The dimensionality of the EMG data was evaluated using different factorization algorithms and tested on different sets of both simulated and experimental EMG data. We combined traditional measures, that may be affected by the presence of noise, with new consistency measures that consider the potential variability of motor modules across strides. The values of the inter-stride consistency measures were much less affected by the amount of noise, thus representing a better way of estimating the actual dimensionality, and clearly demonstrated a systematic trend toward increasing dimensionality of the modules with increasing age. Overall, the findings showed that complexity increases from the neonatal stage to adulthood at multiple levels of the motor infrastructure.

An optimal control framework for the production of limb movements

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Motor control is a fundamental process that underlies all voluntary behavioral responses. Several theories based on different principles (task dynamics, equilibrium-point theory, passive-motion paradigm, active inference, optimal control) account for specific aspects of how actions are produced, but fail to provide a unified view on this problem. We propose a concise theory of motor control based on three principles: optimal feedback control, control with a receding time horizon, and task representation by a series of via-points updated at fixed frequency. By construction, the theory provides a suitable solution to the degrees-of-freedom problem, that is, trajectory formation in the presence of redundancies and noise. We show through computer simulations that the theory also explains the production of discrete, continuous, rhythmic, and temporally constrained movements, and their parametric and statistical properties (scaling laws, power laws, speed/accuracy trade-offs). The theory has no free parameters, and only limited variations in its implementation details and in the nature of noise are necessary to guarantee its explanatory power. An assumption of the model is that the optimal feedback controller is universal (i.e., independent of the task at hand) and thus should not be modified during motor adaptation. We have tested this issue in a force field adaptation experiment. We have shown that the shape of after-effect trajectories is not compatible with a modification at the control level as proposed by compensation/reoptimization models. Accordingly, adaptation would not occur at the control level but at the goal level. An open question is the neural basis of the model. We have trained a neural network to approximate the optimal feedback controller (not only to learn optimal trajectories) and we have shown that properties of one layer of the network closely match those of the primate primary motor cortex.

Which model for interceptive actions?

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Interceptive tasks, such as catching a moving object, pose significant challenges to the human sensorimotor system. Computational models have been instrumental in unraveling the underlying mechanisms and strategies involved in these actions. Several studies presented diverse computational models tailored to interception tasks, including the tau margin theory, outfielder problem, and required velocity model. While these models have contributed valuable insights, they also exhibit limitations. Critically assessing these limitations reveals gaps in our understanding of interception dynamics. For instance, the reliance on specific cues, such as visual size, may oversimplify the richness of sensory information involved in interception. Moreover, the application of optimal control policies may not fully capture the complexity of human interception behaviors, particularly in dynamic and uncertain environments. Additionally, challenges persist in integrating spatial and temporal motor plans effectively to replicate human-like interception accuracy. Furthermore, alternative computational frameworks, such as active inference, propose promising avenues for understanding interception. By acknowledging these limitations and exploring alternative approaches, computational models can advance our comprehension of human interceptive behavior.

Impaired migration and maturation of SST+ GABAergic interneurons in tuberous sclerosis complex

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GABAergic interneurons play a critical role in maintaining the balance of excitatory and inhibitory signals within the brain, essential for proper neural circuit function. The migration of these interneurons from their origin in the ganglionic eminences to their final positions in the cortex is a highly orchestrated process, involving a complex interplay of molecular signals. Disruptions in this migration process are increasingly recognized as underlying factors in various interneuropathies. Impaired migration of GABAergic interneurons can lead to improper integration into neural circuits, disrupting the balance of excitation and inhibition. This imbalance can result in altered network dynamics, manifesting in cognitive and behavioral deficits, as well as increased susceptibility to seizures. Recent investigations into tuberous sclerosis complex (TSC), a genetic disorder characterized by the growth of benign tumors in multiple organs, reveal significant abnormalities in GABAergic interneuron development. Specifically, single-nuclei RNA sequencing and immunohistochemistry analyses have identified profound immaturity and migratory deficits in somatostatin-expressing (SST+) interneurons in TSC. These findings suggest that the impaired migration and maturation of SST+ interneurons contribute to the neurodevelopmental and neurological symptoms observed in TSC patients. This offers potential novel targets aimed at correcting interneuron migration and maturation deficits.

Dysregulation of GABAergic function linked to SST and SST+ interneurons in Tuberous Sclerosis Complex

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Somatostatin (SST) is a neuropeptide known for its inhibitory effects on hormone secretion and neuronal excitability. Recent studies have disclosed its crucial role in modulating synaptic activity. SST exerts its effects through five G-protein-coupled receptors (SSTR1-5), which are widely distributed in the central nervous system. At synapses, SST can influence neurotransmitter release and synaptic function.
Furthermore, it is known that SST also modulates inhibitory synapses by affecting gamma-aminobutyric acid (GABA)ergic transmission in physiological conditions, through the activation of specific SST receptors on presynaptic GABAergic neurons.
Nonetheless, the role of SST and SST+ interneurons in pathology and their contribution to the physiological and pathological neurodevelopment have not been completely unravelled yet. Here, we used the technique of membrane microtransplantation in *Xenopus* oocytes to investigate the effect of somatostatin on GABAergic neurotransmission in TSC and in control tissues.
The potential regulatory role of SST at synapses could highlight its potential as a therapeutic target for neurodevelopmental disorders, such as TSC, that are characterized by GABAergic immaturity, Understanding the precise mechanisms of SST's action at synaptic sites could hence pave the way for novel treatments aimed at restoring synaptic function in these conditions.

Striatal synaptic dysfunctions in a mouse model of Lafora disease

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Research on the mechanisms linking neuronal excitability to synaptic dysfunction in epilepsy has primarily focused on the cortex and hippocampus, often neglecting other brain regions. However, the involvement of the basal ganglia and dopaminergic neurotransmission has been recognized in some epilepsy animal models. Using a murine model of Lafora disease (LD), a rare and fatal form of progressive myoclonus epilepsy, we performed ex vivo patch-clamp recordings to investigate possible alterations in neuronal excitability, synaptic transmission and plasticity in the striatum. Our data revealed time-dependent alterations in cortico-striatal and nigro-striatal synaptic transmission and plasticity in medium spiny neurons of LD mice, potentially linked to dysfunctions in these networks. Remarkably, treatment with either cannabidiol or dopamine was able to reverse the observed synaptic dysfunctions, suggesting potential functional crosstalk between dopaminergic and endocannabinoid signaling in this model.

Gene therapy strategies to rescue synaptic dysfunction in epilepsy

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Epilepsy can be considered an ensemble of diseases of different etiologies, characterized by the spontaneous and unpredictable occurrence of seizures, i.e. by abnormal and hyper-synchronized neuronal discharges from one or several brain areas. An abnormal synchronous neuronal firing occurs when the neuronal networks are irregularly formed or are perturbed by structural, infectious, or metabolic disturbances. The common final effect of these genetic or acquired factors is the lowering of seizure threshold generation, a condition in which a previously normal brain network becomes prone to spontaneously generating seizures. Excitation-inhibition imbalance can modify synaptic activity, leading to brain hyperexcitability. Modulation of synaptic activity can therefore be a strategy to reduce hyperactivity in altered brain circuits.
Through in vitro experiments in primary neuronal cultures and in vivo testing in animal models of epilepsy, we found that enhancing the inhibitory tone of neuronal networks by regulating expression of neuropeptides (e.g NPY and its receptors) or inhibitory neurotransmitter receptors (e.g GABA-A receptors) can indeed rescue dysfunctional synaptic activity and rebalance physiological excitation/inhibition equilibrium.
Overall, our data support the idea that a gene therapy approach that controls hyper-excitability may be an effective strategy to restore physiological neuronal activity and prevent epileptic seizures.

Episodic memory: from the hippocampal-entorhinal circuit to a distributed network

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Episodic memory is defined as the ability to recollect events with their specific spatiotemporal details. This form of memory is critically dependent on the hippocampus and the inputs from the entorhinal cortex. Using the object-place-context recognition task (OPCRT), memory engrams have been identified not only in the hippocampus but also in the lateral entorhinal cortex of the mouse. However, it has been hypothesized that a specific memory is encoded by a distributed network of engram cells, suggesting the involvement of other brain regions in episodic memory. We conducted a brain-wide mapping of neurons activated during the retrieval of OPCR memory, considering the expression of cFos as a marker of neuronal activity. We found that OPCR memory recall specifically increased the activity of associative isocortical regions involved in memory processing, navigation, and decision-making, such as the retrosplenial cortex, the orbitofrontal cortex, and the medial prefrontal cortex. Furthermore, a significant activation was present in the postsubiculum, an input structure to the hippocampal formation that projects to the entorhinal cortex and likely relays inputs from retrosplenial cortex. These results support the idea that episodic memory is stored in a network of functionally connected neuronal ensembles distributed throughout the brain, with the hippocampal formation acting as a central hub for its formation and retrieval.

The cross-talk between Amyloid-β, α7 nicotinic acetylcholine receptors and cyclic nucleotides at the synapse: from physiology to Alzheimer's Disease

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The increase of Amyloid-beta (Aβ) is involved in Alzheimer's disease (AD) pathophysiology, yet when at low concentrations, it ensures memory formation in the healthy brain. Previous studies showed that cyclic nucleotides (CNs) stimulate Aβ production that, in turn, enhances synaptic plasticity and memory via α7 nicotinic acetylcholine receptors (α7nAChR). Indeed, genetic deletion of α7nAChRs in α7-knock out (KO) mice prevents Aβ physiological role, leading to its compensatory increase that induces an AD-like pathology. On the other hand, it is known that increasing CN levels by phosphodiesterase inhibitors (PDE-I) improves cognition in the healthy brain and rescues memory impairment in AD models.
Our study explored whether CNs need Aβ production and function to exert their positive effect. To this end, we used Amyloid Precursor Protein (APP) KO mice, which do not produce Aβ and α7KO mice lacking Aβ physiological receptor.
We found that treatments with PDE4-I roflumilast or PDE5-I vardenafil, increasing cAMP or cGMP respectively, failed to enhance hippocampal synaptic plasticity and memory in young APPKO and α7KO mice compared to WT. Chronic treatment with these PDE-Is also failed to rescue the age-dependent cognitive decline in these models.
Our results suggest that Aβ role is crucial to mediate the effects of CNs on synaptic plasticity and memory, shedding new light on the crosstalk between Aβ, α7nAChR and CNs in physiological conditions and in AD pathophysiology.

Enhancing memory and synaptic plasticity through histone acetylation modulation: implications for Alzheimer's disease therapy

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Neuronal plasticity is crucial for memory formation. Changes in neuronal connections trigger gene transcription programs essential for neuronal plasticity, guided by critical epigenetic modifications. For instance, histone acetylation, a type of epigenetic change, promotes gene transcription leading to memory formation. Specific histone acetyltransferases (HATs) enhance memory, while histone deacetylases inhibit it. Disruptions in these processes are likely to contribute to memory loss in Alzheimer’s Disease (AD). We found reduced levels of memory related HATs associated with decreased histone acetylation (H3K4, H3K9 and H3K14) in AD brains compared to control healthy individuals. An electric shock eliciting associative memory formation increased their acetylation, and upregulated memory-related genes, CREB, pCREB, Arc, c-Fos, and BDNF in C57Bl6 mice. However these effects did not occur in the hTau/Mapt-KO mouse model of AD. Most importantly, OA57, a small molecule enhancing acetylation of H3K4, H3K9 and H3K14 in a cell free assay, restored normal levels of histone acetylation, and improved both memory and synaptic plasticity in AD mouse models. Altogether these findings suggest that increasing histone acetylation in neurons may be beneficial against AD.

A vicious circle among Interleukin-1β, Aβ and Tau affects synaptic function and memory

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We recently developed a mouse model of multiple Herpes simplex virus type-1 (HSV-1) reactivations within the brain induced thermal stress (TS), exhibiting increased brain levels of the pro-inflammatory cytokine interleukin 1 (IL-1β), along with a progressive accumulation of amyloid-β (Aβ) and tau proteins.
Exploiting this model, we investigated the crosstalk among IL-1β, Aβ and tau in synaptic and memory deficits observed in infected mice. The casual role of IL-1β was assessed by blocking the IL-1 receptor with Anakinra that rescued all indices of synaptic dysfunction after 2TS. HSV-1-infected $APP^{-/-}$ and $Tau^{-/-}$ mice undergone 2TS exhibited IL-1β mRNA levels that were higher than in mock-infected transgenic mice (1.6- and 1.4-fold induction, respectively, $p<0.05$) but significantly lower than in WT mice (-32% and -43% in HSV-1-infected $Tau^{-/-}$ and $APP^{-/-}$ mice, respectively). Accordingly, transgenic mice showed milder synaptic deficits than WT mice: LTP at the hippocampal CA3-CA1 synapses in HSV-1- vs mock-infected mice was 73.7±6.7% vs 98.6±10.1% in $Tau^{-/-}$ mice, 69.5±5.6% vs 94.6±6.4% in $APP^{-/-}$ mice and 47.3±6.4% vs 98.0±12.4% in WT ones.
Our findings reveal a prominent role of IL-1β in synaptic failure when the accumulation of misfolded proteins is still limited. The lower levels of IL-1β and the milder synaptic deficits observed in $Tau^{-/-}$ and $APP^{-/-}$ mice indicate the critical role of the vicious circle established among IL-1β, Aβ and tau in disrupting synaptic function.

Development of adaptive locomotion in children with Cerebral Palsy

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Early life is an important period for the development of the spinal locomotor circuitry. Developmental motor disorders (such as cerebral palsy, CP) can compromise exploration and flexibility of behaviour, making it crucial to understand the specific locomotor impairments associated with pathology. We examined several adaptive locomotor tasks in children with CP (6mo – 12yrs). The study demonstrated that 1) the development of spinal locomotor drives is significantly delayed until the onset of unsupported walking, rather than following a genetically predetermined time course, 2) a lack of adaptation (a preservation of FW basic motor modules) and a lack of differential control of right and left limb muscles when performing other directional movements, such as walking sideways or backward, 3) an absence of the adaptable muscle module timed to the voluntary task (lift-off) during obstacle avoidance. Overall, the findings support the idea that complex locomotor movements can be used for more comprehensive diagnosis of CP as well as the ability of a child to cope with novel task requirements may represent a useful rehabilitation tool to improve the locomotor performance. The findings also lend credence to the notion that children with CP require early therapies to promote the locomotor function.

The greater force exerted by dominant muscles is driven by a higher excitatory input to motoneurons at various contraction intensities

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Understanding the physiological mechanisms underlying limb dominance is crucial for comprehending the asymmetrical motor control observed in humans. However, the neuromuscular determinants governing the dominance of one limb over its contralateral still need further exploration.
High-density surface electromyography (HDsEMG) was applied on twenty participants performing maximal voluntary contractions, trapezoidal ramps at 35% and 70% of maximal voluntary force (MVF) and sustained contractions at 10%MVF. Motor unit discharge and recruitment properties were assessed in biceps brachii from the dominant and non-dominant sides. The proportion of common synaptic input to motoneurons was estimated with coherence analysis during sustained tasks.
A greater MVF was found in the dominant limbs (+9%, $p=0.001$) with similar recruitment and derecruitment thresholds ($p>0.05$). A higher motor unit discharge rate was observed on the dominant side (+12%, $p<0.001$), along with a greater proportion of common synaptic input (+22%, $p=0.002$). The relationship between force exertion and rate coding during the ramp phase was similar between dominant and non-dominant limbs ($p>0.05$).
The similar association between the excitatory input and the mechanical output confirmed that the higher muscle strength of the dominant side stems from a greater synaptic input to the motoneurons. Limb dominance is primarily explained by differences in motor unit rate coding rather than recruitment properties.

Atm deficiency is associated with motor impairment and dysfunction of cerebellar circuitry in mice

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The *ATM* gene encodes a Ser/Thr kinase, a phosphoinositide 3-kinase-related protein kinase family member, crucial for the response to DNA double-strand breaks. Mutations of the *ATM* gene cause Ataxia telangiectasia (A-T), a progressive childhood neurodegenerative disease with early onset cerebellar ataxia, indicating its important role in the nervous system. The precise mechanisms by which Atm affects the cerebellar circuitry remain unclear. This study was aimed at elucidating the role of *ATM* in the cerebellum, focusing on its impact on synaptic transmission. We used a novel murine model with a catalytically inactive ('kinase-dead';-KD) enzyme only in the central nervous system. We employed a combination of behavioral, electrophysiological, and immunohistochemical approaches to investigate Atm^{KD} mice. Atm^{KD} mice showed significant motor impairment in the balance beam and in the footprinting test at 2 months. These motor impairments were associated with alterations of cerebellar synaptic transmission. Specifically, whole-cell patch-clamp recordings from cerebellar Purkinje cells (PC) revealed a significant increase in intrinsic excitability in Atm^{KD} mice. Aditionally, the short-term plasticity of the climbing fiber-PC synapse was reduced.
Our findings suggest that ATM's functions extend beyond DNA repair, influencing the maintenance of normal cerebellar function, likely through its role in modulating intrinsic excitability and synaptic plasticity.

Artificial intelligence allows for the automatic identification of taste fungiform papillae

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Taste sensory cells of fungiform papillae (FPs) are the initial component of the process of perception and discrimination of stimuli involved in food nature and quality. The number of fungiform papillae varies greatly among individuals. Reductions of their numbers not only diminish identification of nutrient-rich food and avoidance of noxious substances, but also the joy of consuming tasty foods and, consequently, the pleasures of social eating.
Here we present direct measures to accurately and automatically identify FPs with an automated method based on a deep learning architecture (U-Net) for image segmentation. Our approach uses a convolutional neural network to analyze images of the tongue, effectively distinguishing FPs from the surrounding tissue. The U-Net model is trained on a diverse dataset, ensuring it can handle variations in papillae morphology and distribution. The results show a robust performance of the method.
Our study provides significant advancements in taste and nutrition research, offering a quick, precise and efficient method for automated FPs identification. Future work will focus on refining the model and exploring its application in diagnostic tools.

Altered brain networks and auditory dysfunctions in 3×Tg-AD mice underlying early noise-induced memory deficits

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The clinical association between hearing impairment and cognitive decline has been widely documented. However, the complex pathophysiological relationship between auditory deprivation and dementia remains to be fully defined. Our hypothesis is that auditory and cognitive degeneration share common pathogenetic pathways and that cochlear input can impact cognitive processes impinging on the neuronal networks involving hearing and cognitive brain structures, such as the auditory cortex (ACx) and hippocampus (HP). We previously demonstrated that noise-induced hearing loss (NIHL) in an animal model of Alzheimer's disease (3×Tg-AD mice) before the neurodegenerative phenotype is manifested can accelerate memory deficits, causing persistent synaptic and molecular alterations in ACx and HP (PMID: 34699347). In this study we further investigated possible baseline auditory dysfunctions in 3×Tg-AD mice, by using electrophysiological, morphological and molecular analyses. Our results provided evidence of synaptic alterations in both the cochlea and the ACx of 3×Tg-AD mice compared to wild-type strain, with changes in the afferent/efferent cochlear innervation and an imbalance of the excitatory/inhibitory network in the ACx. Collectively, these data demonstrate a higher vulnerability of the auditory system in 3×Tg-AD mice, probably underlying the early cognitive impairment induced by NIHL.

Sensory afferents from the face modulate cortical sensorimotor integration with topographic specificity and influence face expressions recognition

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In the cranial district, sensorimotor integration is important in adjusting movements modulating primary motor cortex (M1) excitability and its impairment may alter face expressions recognition (FER). In this light, individuals with malocclusion (IM) represent the perfect model to explore how sensory afferents alterations can influence sensorimotor integration and FER. In the present study we measured 1) short-interval intracortical inhibition (SICI) and short-afferent inhibition (SAI) in the M1 face and masticatory representations areas, using transcranial magnetic stimulation protocols, and 2) FER task performance, in 18 healthy subjects (HS) (24.56±1.02 years old) and in 18 IM (27.36±1.39 years) before and after 1 month from surgery. In IM we found a significant reduction of SICI ($p=0.034$) and SAI ($p=0.007$) in the masticatory, but not face M1. IM also exhibited deficits in FER before surgery ($p=0.033$), which improved after surgery ($p=0.015$). Results evidence that an alteration of sensory afferents modulates sensorimotor integration in masticatory M1, with topographic specificity. The altered sensory information from face not only influences motor inhibitory control but also FER. In conclusion, the integration of sensory inputs with motor outputs depends topographically on the type of sensory afferents stimulated as well as on the target muscle and its respective representation area in M1. This process plays also a role in FER.

Tamoxifen impaired the lithium-induced increased cilium length and necroptosis reduction

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Lithium is often used to treat some mental illnesses; however, several adverse effects have been reported including acquired nephrogenic diabetes insipidus (NDI). Tamoxifen, an estrogen receptor modulator, mitigates the onset of lithium-induced NDI.
Here, *ex vivo* and *in vivo* experiments revealed that treatment with Tamoxifen impaired the lithium-induced reduction in necroptosis in the renal inner medulla. Moreover, lithium administration significantly decreased the expression of RIPK3 and MLKL which are key players in controlling necroptosis. Conversely, these effects were prevented by Tamoxifen. *In vitro* experiments, in MCD4 cells, confirmed those findings. Also, a decrease in the expression of BID and an increase of beclin, selective markers of apoptosis and autophagy respectively, were found as well. Indeed, lithium exposure increased cell proliferation, transepithelial electrical resistance (TEER), and the primary cilium length as assessed by the super-resolution stimulated emission depletion (STED) microscopy. The lithium effects were prevented by Tamoxifen treatment.
These data revealed for the first time that exposure of renal collecting ducts to lithium significantly reduced the expression level of selective necroptosis markers. In addition, lithium promotes inappropriate cell proliferation possibly associated with increased transepithelial electrical resistance and elongation of the primary cilium. Tamoxifen prevents all these effects.

Do visual and vestibular perceptual biases mutually compensate during the execution of a self-motion visuo-vestibular task?

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Gravity affects the motion of all objects we encounter in daily life by accelerating them toward the center of the Earth. Its expectation has been shown to influence human perceptual judgments. In fact, downward-moving visual targets are perceived as faster than upward-moving ones, and a directional asymmetry emerges in the sensitivity of speed judgement of vertically-moving objects, both indicating that gravity is an internalized prior. Similar results are observed during vertical self-motion: higher sensitivities are noted during the vestibular perception of downward compared to upward motion. It remains unclear whether the internalized gravity prior also affects the vestibular perception of vertical self-motion duration and how such prior influences visual and vestibular integration. To investigate this, we designed a task where participants estimated the duration of motion along the Earth's vertical axis using visual targets (VI), passive whole-body movements (VE), or both (VV), in three separate blocks in which participants compared the durations of two stimuli with opposite motion directions.
In both visual and vestibular blocks, downward-moving stimuli were perceived as faster than upward-moving ones, confirming the predictions of perceptual biases. However, when both visual and vestibular information were available, these perceptual biases were canceled, suggesting that the integration of information leads to a more accurate estimation of reality.

Ab initio definition of a functional network involving transthyretin, carnosine and copper with (patho)physiological implications in the familial amyloid polyneuropathy (TTR-FAP) rare disease

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Transthyretin (TTR) is a carrier protein for thyroid hormone T4 and retinol in blood and CSF. In the rare Familial Amyloid Polyneuropathy (TTR-FAP), TTR mutations enhance amyloidosis causing polyneuropathy and autonomic dysfunction. Among assessed anti-amyloid disruptors, carnosine (CAR, β-alanyl-L-histidine) is a homeostatic dipeptide in excitable tissues/cells, deriving from both dietary intake and biosynthesis. As chelator CAR influences homeostasis of divalent ions including copper (Cu) i.e. a destabilizing agent in diverse amyloidosis including TTR-FAP. Here, we explore *ab initio* the interactions between CAR and the wild type TTR or a cohort-specific Phe84Leu mutation causing amyloidosis in TTR-FAP patients. By molecular docking/dynamics models, we describe different interactions between CAR and wild type or mutant TTR eliciting differential interference with the aggregation process. We show different TTR-CAR structure/function relationships implied by Phe84Leu mutation, also comparing CAR with overt anti-aggregation molecules. In parallel, analyses in blood from a cohort of TTR-FAP patients brought to light, unprecedentedly, statistical correlations among individual Phe84Leu TTR levels, Cu content in plasma/serum fractions, circulating ceruloplasmin and serum CNDP1 (i.e. CAR degrading enzyme). Taken together, the data support the novel hypothesis of TTR/CAR/Cu as functional triad involved in the dysregulations that exacerbate amyloidosis in the TTR-FAP rare disease.

Function role of Neuroglobin trafficking in mitigating ER-stress response in human neuron derived-cells

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Chronic or dysfunctional responses to endoplasmic reticulum (ER) stress contribute to neuronal degeneration in neurodegenerative proteinopathies. The intracellular accumulation and the extracellular trafficking of neuroglobin (NGB) play protective roles under stress conditions, but the globin's implications in ER stress response remains unexplored. Here, we investigate the effects of intracellular and extracellular NGB on ER-stress induced by Brefeldin-A (BFA) in undifferentiated and differentiated neuron derived SH-SY5Y cells. In undifferentiated cells, results indicate that NGB overexpression prevents apoptosis, improves cell viability, and preserves mitochondrial polarization after BFA stimulation. Similarly, the treatment with NGB-enriched conditioned medium (CM) before BFA exposure improves cell viability and reduces apoptotic death, by attenuating ER-stress response. Results, obtained in differentiated SH-SY5Y with a more mature neuron phenotype, demonstrate that overexpressed NGB completely preserves neuritic length and number, while NGB-enriched CM partially restored neuritic structure after BFA treatment, mitigating ER-stress response. Taken together, these data indicate that intracellular and extracellular NGB can alleviate the chronic activation of ER-stress response and exert a neuroprotective role, suggesting that modulation of NGB levels and/or the delivery of extracellular NGB could be a promising strategy for treating neurodegenerative proteinopathies.

Targeting the urokinase-type plasminogen activator receptor system to improve visual function in the rd10 mouse model of retinitis pigmentosa

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Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies characterized by progressive and irreversible loss of vision due to rod and cone degeneration. Despite the primary genetic etiology of the disease, neuroinflammation is now considered a hallmark of many chronic neurodegenerative disorders including RP. The urokinase plasminogen activator (uPA) and its cognate receptor (uPAR) system has been recently suggested as a possible contributor to the onset of inflammatory mechanisms characterizing several retinal disorders. Here, we investigated if N-19004, a novel synthesized uPAR antagonist, may reduce neuroinflammation in a mouse model of RP thus slowing down the disease progression. To this aim, we used rd10 mice in which rods degenerate maximally between postnatal day (PD) 20 and PD25. The efficacy of N-19004 was investigated using optical coherence tomography, electroretinographic recordings, Western blot and immunofluorescence. In rd10 mice, N-19004 administration reduced both Müller cell gliosis and up-regulated levels of inflammatory markers, and exerts major anti-apoptotic effects. Rescue from retinal cell degeneration was accompanied by improved retinal function. In summary, our study demonstrates that counteracting neuroinflammation may ameliorate retinal function even in genetically-determined retinal dystrophies such as RP, and supports the involvement of the uPA/uPAR system as a possible regulator of neuroinflammation and retinal function.

Downregulation of vasopressin-AQP2 pathway explains the secondary NDI associated with cystinosis: *in vivo* and *in vitro* evidence

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Cystinosis, a lysosomal storage disorder, is caused by mutations in *CTNS* gene, encoding for cystinosin. Cystine accumulates throughout the body, primarily affecting the kidneys, leading to severe tubular dysfunction and glomerular damage. Secondary Nephrogenic Diabetes Insipidus (NDI) has been reported as a complication of cystinosis, due to the resistance to vasopressin, a key hormone regulating collecting duct water reabsorption through the water channel Aquaporin-2 (AQP2). The lack of a cystinotic collecting duct *in vitro* model has limited the investigation of the mechanisms causing the defective urine concentrating ability. In this study, we established the first CRISPR/Cas9 collecting duct *in vitro* model knocked out for *CTNS*. These cells showed a strong reduction in AQP2 expression, and no increase in osmotic water permeability in response to desmopressin, likely due to reduced AQP2 expression. Treatment with chloroquine, an autophagy inhibitor, resulted in a significant increase in AQP2 expression, indicating that in KO *CTNS* cells, AQP2 downregulation is mediated by enhanced autophagic degradation. In four cystinosis patients, AQP2 excretion, a biomarker for collecting duct responsiveness to vasopressin, did not significantly increase after desmopressin treatment along with no significant increase in urine osmolality. Together, these data indicate that secondary NDI in cystinosis is due to a defect in the vasopressin-AQP2 axis mainly due to AQP2 downregulation.

Protective effects of the extra virgin olive oil phenol compound hydroxytyrosol against palmitate-induced organelle stress and insulin resistance

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Hepatic steatosis is a dysmetabolic disease linked to obesity. Recent studies have shown that bioactive molecules found in healthy foods like extra virgin olive oil (EVOO) polyphenols can improve cellular function. In this work we investigated the protective effects of hydroxytyrosol (HT) against palmitate (PA) exposure in liver cells.
HepG2 were treated with different doses of PA (100, 250, 300, 500µM), or HT (50 and 100µM). Co-incubation of PA and HT was also performed. The analyses include the assessment of cellular viability and morphology; ROS production; cytochrome oxidase (COX) activity; lipid depots and mitochondrial network in cells. The contents of the main proteins involved in mitochondrial dynamics, apoptosis, and ER-stress, as well as the ability of cells to respond to insulin signals, were also detected.
Our results evidenced that PA exposure increased lipid depots, oxidative stress, mitochondrial dysfunction and fragmentation, ER-stress, cell death, and insulin resistance. PA/HT coincubation partially reverted PA effects, improving mitochondrial function, and reducing lipid depots and ER-stress markers. In addition, HT also restored the balance between fusion and fission processes altered by PA, accompanied to organelle’s network reorganization, and with an improvement of insulin sensitivity.
In conclusion, our analyses evidenced that HT exposure reverts PA-induced hepatic damages, suggesting its possible protective effect in the control of steatosis onset.

Developmental and epileptic encephalopathy-associated mutations cause constitutive opening of the Kv7.2 channel inner pore gate

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Kv7.2/3 channels, encoded by members of the KCNQ gene family, are responsible for the generation of the M-current, a slowly activating and deactivating potassium conductance that plays a critical role in neuronal excitability. Variants in the KCNQ2 gene have been linked to phenotypically heterogeneous neurological diseases, making this protein a prime candidate for drug development. While most mutations in the Kv7.2 pore reduce the M-current, a new class of Gain-of-Function (GoF) variants, affecting residues near the intracellular side of the inner gate (IG) of the pore, have been recently identified. Here, we investigate the impact of three IG mutations (G313S, A317T, L318V) on the structure of the wild-type (WT) Kv7.2 protein using all-atom molecular dynamics (MD) simulations on the microsecond timescale. We analyzed both closed and open channel configurations. All the examined mutations induced a widening of the gate of the closed configuration with respect to the WT protein, consistent with a GoF effect revealed by electrophysiological experiments, while they showed no effect on the open conformation. In conclusion, we provide an atom-detailed structural characterization of different Kv7.2 variants, crucial to understand the molecular mechanisms underlying the different phenotypes.

Unveiling the role of primary cilia: insights into cAMP signaling dynamics and GPCR functionality using advanced imaging techniques

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The primary cilium is a distinct cAMP microdomain that expresses a specific subset of cAMP-linked GPCRs. However, our understanding of the signalling mechanisms mediated by these GPCRs and how cells distinguish cAMP signalling events originating within the cilium versus those originating in the cell body remains incomplete. Here, we used a cilium-targeted FLIM-based cAMP sensor (Arl13b-EPAC-SH189) to study whether β-adrenergic signalling takes place directly in primary cilia of RPE-1 cells. We combined this biosensor with an over-expressed phosphodiesterase, PDE8A, to buffer the contribution of cAMP signals emanating from the cell body. When control cells were stimulated with 1.2 nM isoprotenerol (Iso), we observed the same pattern of cAMP production using either the cytosolic or the ciliary FLIM cAMP sensor. When we clamped cytosolic cAMP using the PDE8 construct, treatment with Iso did not alter ciliary cAMP until the addition of a potent PDE8 inhibitor, indicating the absence of independent ciliary cAMP production and diffusion of cAMP from the cytosol. To track active Gαs-coupled GPCRs in the cilium, we monitored ciliary accumulation of an mVenus-tagged miniGs construct (mGs). Iso stimulation did not result in accumulation of the mG in the ciliary domain, further suggesting that Iso-stimulated cAMP originates from outside the cilium. In conclusion, our study illustrates the potential utility of two new approaches for dissecting the signalling functions of ciliary GPCRs.

AI-based prospective repurposing investigations for the identification of new promising vasopressin V2 receptor ligands

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The vasopressin V2 receptor (V2R) controls renal water balance. Besides water reabsorption, V2R is involved in abnormal cell proliferation, cancer, and cyst growth in polycystic kidney disease. Here, we performed an inverse screening of a large collection of known drugs to identify novel V2R ligands that might modulate different receptor-mediated effects.
The inverse screening campaign was run by using PLATO, a target fishing platform. Structure-based studies were also carried out**.**
Our prospective repurposing studies identified five promising drugs as potential V2R ligands. Interestingly, FRET and functional analysis, in renal collecting duct MCD4 cells, revealed that one of them (coded as F2544) at 1nM concentration significantly reduced the DDAVP-dependent cAMP production and the DDAVP-induced water reabsorption, with effects comparable to tolvaptan, that is a well-established V2R antagonist. In this respect, an *in-depth* computational investigation showed a nice overlap of the molecular interaction fields generated from the binding sites of V2R and F2544. Molecular docking simulations returned a promising posing and scoring of F2544 in the V2R binding site.
The present findings identify for the first time new V2R ligands by applying an AI-based approach. By combining *in-depth* computational investigations and functional studies, F2544 was prioritized for being repurposed for treating diseases associated with abnormal V2R signaling.

Neural stem cell-derived extracellular vesicles preserve brain plasticity in experimental models of metabolic and neurodegenerative disorders

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Neural stem cells (NSC) represent a fundamental resource for brain plasticity and repair through generation of newborn neurons and release of neuroplasticity-related molecules. NSC are also a plentiful source of extracellular vesicles (EVs), nano-sized membranous structures carrying small RNAs, DNA, lipids and proteins. However, an exhaustive characterization of both the NSC-derived exosomal cargo and their functional role in modulating brain plasticity remain elusive. Recently, we discovered the potential therapeutic effect of mouse NSC-derived EVs (exo-NSC) in a well-characterized model of brain insulin resistance (BIR)-dependent cognitive decline. Exo-NSC act on both NSC and mature neurons by restoring insulin and BDNF-related pathways, respectively, preserving the neurogenic niche and counteracting memory deterioration. Alzheimer's disease (AD) is a disabling neurodegenerative disorder associated with progressive development of neuroinflammation and BIR. Chronic intranasal administration of mouse exo-NSC counteracted the development of memory deficits in both male and female 3×Tg-AD mice at early, middle, and late stages of the disease (i.e., 6, 9, 12 months). Moreover, exo-NSC administration reduced the deposition of amyloid-β and neuroinflammation in the hippocampus of AD mice. Understanding the therapeutic potential of exo-NSC in neurological disorders associated with cognitive decline may pave the way for new pioneering approaches against neurodegenerative disorders.

Time-dependent phenotypical changes of microglia drive alterations in synaptic transmission in acute hippocampal slices

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It is well known that microglia actively regulate synaptic function in the brain. Although much of our understanding on microglia-mediated synaptic regulation derives from acute brain slices, it is still uncertain i) to what extent slices preparation and maintenance influence microglia function and ii) whether these changes affect synaptic transmission. In this study, we examined the impact of acute slice resting time on hippocampal CA1 microglia, by assessing microglia properties at distinct time intervals. We report that microglia undergo time dependent morphofunctional and transcriptional changes, including a decrease in branches number and speed. Furthermore, microglia acquire a reactive phenotype, characterized by increased amplitude of outward K^+ currents and altered expression of TNFa, CX3CR1 and P2Y12R.

We also examined time-dependent changes of excitatory postsynaptic currents (sEPSCs) in CA1 pyramidal neurons from acute hippocampal slices, reporting time-dependent decrease in both amplitude and frequency. Noticeably, sEPSCs amplitude decrease was absent in slices prepared from PLX 5622 microglia-depleted mice.

Our findings highlight possible causal relation between microglia phenotypic changes in slices and concomitant synaptic changes, pointing to interplay mechanisms, whose understanding is crucial for unraveling microglia-neurons interactions in nature. Furthermore, they emphasize the potential issues associated with experimental time window in ex vivo samples.

High fat diet reduces hippocampal dendritic spine density by altering S-palmitoylation of synaptic proteins

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Brain's structural plasticity is the ability to change its physical structure in response to environmental stimuli. This dynamic process involves the formation of new neurons, synaptogenesis, dendritic growth and pruning, and axonal sprouting. We previously demonstrated that high-fat diet (HFD) affects synaptic plasticity by altering protein S-palmitoylation, a post-translational modification regulated by zDHHC enzymes. By palmitoyl-proteome analysis, we identified 173 hippocampal proteins whose S-palmitoylation was affected by HFD. Bioinformatic analysis revealed several proteins involved in structural plasticity that were aberrantly palmitoylated. In the hippocampi of HFD mice, we observed increased S-palmitoylation levels of RAB3A, Debrin, Dynamin-1, RAC1, and GAP43. This increase was associated with higher expression levels of zDHHC3, zDHHC7, zDHHC9, and zDHHC24 enzymes, suggesting an alteration in the regulatory mechanisms of synaptic function. Furthermore, this increase in synaptic protein palmitoylation was accompanied by a strong reduction in dendritic spines in the CA1, CA3, and dentate gyrus regions of the hippocampus. Of note, the reduction of dendritic spines was counteracted by intranasal administration of the S-palmitoylation inhibitor 2-bromopalmitate. Our findings identified novel potential targets of S-palmitoylation and revealed new insights into the molecular mechanisms underlying the impact of nutrient-related signals on brain structural plasticity.

Patch-clamp and diamond micro-electrode arrays (µ-D-MEA) recordings to monitor the action of exogenous α-synuclein on dopaminergic neurons' electrical activity and dopamine release

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Parkinson's disease is a motor and cognitive neurodegenerative disorder associated with the spreading of α-synuclein aggregates that induce the progressive loss of dopaminergic neurons (DA) in *Substantia Nigra pars compacta (SN)*. Growing evidence suggest that extracellular α-synuclein oligomers represent the most neurotoxic species and thus may be involved in the early stages of PD onset.
By merging conventional electrophysiological (patch-clamp) together with diamond micro-electrode arrays (µ-D-MEA) recordings, we quantified the effect of extracellular α-synuclein oligomers respectively on firing discharge and quantal dopamine release in midbrain DA neurons. After 48 hours exposure to α-synuclein (1 µM), the spontaneous firing discharge was severely impaired and decreased from 3.7 to 1.7 Hz. Interestingly, the firing discharge was restored to control values by ω-conotoxin MVIIC administration, suggesting that α-synuclein up-regulates Cav2.1 and Cav2.2 calcium channel isoforms. To test this hypothesis and assess whether Cav2.1 and Cav2.2 potentiation could also exert an effect on DA release, we measured the exocytotic activity of DA neurons by means of µ-D-MEAs. We found that α-synuclein drastically increased the occurrence of quantal exocytotic events, from 0.25 to 6.96 Hz. Thus, we can conclude that exogenous α-synuclein increases DA release through Cav2 current potentiation and that this effect interpheres with the spontaneous firing discharge of DA neurons.

Evaluating early synaptic changes in a model of synucleinopathy before overt behavioral alterations

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In synucleinopathies, the accumulation of α-synuclein aggregates is associated with neurodegeneration, neurotoxicity, and proinflammatory responses, even at low concentrations, underscoring their pivotal role in the pathogenesis of Parkinson's disease (PD). The precise mechanisms underlying α-synuclein-related neurotoxicity—whether arising from dispersed oligomeric species or from α-synuclein species in equilibrium with more aggregated forms—remain unclear.
To address this, we utilized a rat model of synucleinopathy induced by intrastriatal injection of α-synuclein, aiming to elucidate early events that precede synaptic and motor symptoms. Electrophysiological assessments and behavioral assays revealed several early alterations in α-synuclein-injected rats, evident as early as 12 weeks post-injection. These included reduced motor activity, anxiety-like behavior, impaired bidirectional striatal long-term synaptic plasticity, and decreased spontaneous glutamate release in the striatum.
Experimental strategies to counteract these changes will be discussed, aiming to provide a foundation for novel molecular targets and drug treatment strategies. These strategies are intended to mitigate or delay early damage at cortico-striatal terminals induced by α-synuclein, thereby counteracting the pathophysiological processes underlying the onset of early symptoms in PD.

New strategies to counteract dopaminergic degeneration: *in vitro* and *in vivo* neurotrophic properties of the first positive allosteric modulator for metabotropic glutamate receptor 3

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Parkinson's Disease (PD) is a neurodegenerative disorder marked by the progressive loss of dopaminergic neurons in the nigrostriatal circuit. Currently, available pharmacological treatments offer only symptomatic relief with adverse effects. Within this framework, indirect evidence has indicated that activating metabotropic Glutamate Receptor 3 (mGluR3) may exert neuroprotective effects in animal models of PD, but selective agonists/ligands for this receptor were lacking until now. Recently, a groundbreaking development has emerged in the form of a new Positive Allosteric Modulator (PAM) specifically designed for mGluR3, overcoming past limitations. Our study shows for the first time that the novel mGluR3 PAM protects the human neuroblastoma cell line SH-SY5Y against cell death induced by exposure to the dopaminergic toxin 6-hydroxydopamine through the modulation of key signaling pathways, such as mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3K)-Akt pathways. Moreover, *in vivo* experiments revealed that administration of the original mGluR3 PAM upregulates the expression of crucial neurotrophins, such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), while influencing MAPK/ERK and PI3K-Akt cascades in the mouse brain. Finally, our inedited findings show the therapeutic potential of the novel mGluR3 PAM as a disease-modifying treatment against PD.

Blocking dopamine D3 receptors enhances hippocampal synaptic plasticity and memory via post-synaptic mechanisms

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Dopamine D3 receptors (D3Rs) contribute to various brain functions, including cognition. Although predominantly in dopaminergic regions, D3Rs are also expressed in the hippocampus where they regulate glutamatergic transmission. We have recently found that blocking D3Rs with the antagonist NGB2904, or through genetic deletion in D3 knockout mice, enhanced hippocampal long-term potentiation and memory in young animals.
Here we studied whether this pro-cognitive effect was mediated by pre- and/or post-synaptic mechanisms. We performed electrophysiological experiments of field recordings and whole-cell patch clamp to assess pre- and post-synaptic forms of transmission and plasticity; western blot (WB) to evaluate the expression of plasticity-related proteins; and electron microscopy to investigate the hippocampal distribution and sub-localization of D3Rs.
We found that D3R blockade improved AMPAR-mediated currents, mEPSC amplitude, and basal synaptic transmission without affecting afferent volley. Conversely, presynaptic forms of plasticity, like post-tetanic potentiation and paired-pulse facilitation, remained unchanged. WB analysis revealed an increased expression of the post-synaptic proteins PSD95, phospho(p)GluA1, and p-CREB. Quantitative electron microscopy showed a higher D3R expression in post-synaptic dendrites compared to axon terminals.
Taken together, our findings suggested that D3R blockade enhanced synaptic plasticity and memory through post-synaptic mechanisms.

A pH-sensitive closed-loop nanomachine to control hyperexcitability at the single neuron level

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Epilepsy is a frequent disease affecting about 1% of the general population and characterized by recurrent seizures. Despite the success of anti-epileptic drugs, nearly one-third of patients do not respond to available therapies. Many drug-resistant epilepsies have a local origin, triggered by the presence of a focus of hyperexcitable neurons. During seizures, neurons accumulate protons leading to intracellular acidic shifts. Based on the idea that the intracellular acidosis can be considered a precocious and reliable proxy of upcoming paroxysmal activity at the single neuron level, we developed a closed-loop chemo-optogenetic nanomachine, named pH sensitive Inhibitory Luminopsin (pHIL). pHIL is composed of a light generator, a fluorescent sensor of intracellular pH (E^2GFP), and optogenetic actuator (halorhodopsin) for silencing neuronal activity. pHIL undergoes bioluminescence resonance energy transfer between luciferase and the first E^2GFP acceptor which activates halorhodopsin by fluorescence resonance energy transfer. In HEK293 cells, pHIL was effective in inducing hyperpolarization and aborting activity elicited in neuronal networks by the administration of convulsants. Moreover, in-vivo expression of pHIL in the hippocampus was effective in decreasing seizures in a murine model of epilepsy. The results indicate that pHIL represents a promising gene therapy strategy for drug-resistant epilepsy.

Mitochondrial influence on performance fatigability: considering sex variability

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Females generally demonstrate higher fatigue resistance than males during isometric contractions. However, when it comes to single-limb dynamic exercises, the intricate interplay between
performance fatigability (PF), cardiovascular responses, and muscle metabolism in relation to sex differences remains underexplored. This study investigates how sex affects the relationship between muscle oxidative characteristics and the development of PF during dynamic single-leg exercise. Twenty-four young healthy participants (12 males *vs.* 12 females) performed a constant-load single-leg
knee extension task (85% peak power output; 60 rpm) to exhaustion (TTE). Neuromuscular assessments via transcranial magnetic and peripheral stimulations were conducted pre- and post-exercise
to evaluate central and peripheral factors of PF. Vastus lateralis muscle biopsies were obtained for mitochondrial respiration and immunohistochemistry analyses. Participants performed similar total work (30±12 *vs.* 26±14kJ, p=0.52) and TTE
(384±141 *vs.* 369±153sec, p=0.70); after the TTE, females' maximal isometric voluntary contraction (MVIC: -36±13 *vs.* -23±10%, p=0.006) and resting twitch (RT: -65±9 *vs.* -40±24%, p=0.004) force declined less. No differences were observed in supraspinal neuromuscular factors (p>0.05). During exercise, females relied more on increasing HR than males (p=0.030). Although fiber type composition was similar (type I: 48±12 vs. 58±13%, p=0.06), males had lower mitochondrial net oxidative capacity (61±30 vs. 89±37, p=0.049) and higher Complex II contribution to maximal respiration (CII; 59±8 vs. 48±6%, p<0.001), which correlated with the decline in MVIC (r=-0.74, p<0.001) and RT (r=-0.60, p=0.002). Females display greater resistance to PF during dynamic contractions, likely due to their superior mitochondrial efficiency and lower dependence on mitochondrial CII activity.

Coincidence detection between apical and basal dendrites drives STDP in cerebellar Golgi cells

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Cerebellar Golgi cells (GoCs) segregate the parallel fiber (pf) and mossy fiber (mf) inputs on their apical and basal dendrites, respectively. Recently, computational modelling predicted that this anatomical arrangement, coupled with a specific ionic channel localization, could be instrumental to drive spike-timing dependent plasticity (STDP) at mf-GoC synapses. Here, we have performed GoC patch-clamp recordings in mouse cerebellar slices to investigate the impact of temporally correlated inputs conveyed through mfs and pfs. Repeated mf-pf pairing induced a typical bidirectional Hebbian-STDP, with spike-timing long-term potentiation or depression (st-LTP or st-LTD) occurring when action potential (AP) elicited by pf stimulation followed or preceded the activation of mf synapse, respectively. STDP was restricted within a ±50 ms time window and required AP back-propagation from apical to basal dendrites, NMDA receptor (NMDAR) activation at mf-GoC synapses, and intracellular calcium changes. Moreover, STDP induction occurred specifically when pairing mf-pf stimulations at 4 Hz. In aggregate, GoCs, thanks to two sets of dendritic projections (that allows pf-mf input combinations) and synaptic NMDARs (that allow voltage-dependent Ca-entry in basal dendrites), might operate as *circuit coincidence detectors* between the granular and molecular layers. This detector is tuned on the theta-band that provides a preferential frequency range for cerebellar computation and learning.

NOD-1 activation increases the spontaneous activity and the I(f) current of murine sinoatrial node cells and alters their response to sympathetic stimulation

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NOD1, an intracellular innate immune receptor, is implicated in inflammation and cardiac dysfunction. The activation of NOD1 has been associated with conditions such as sepsis and earlystage heart failure which are characterized by tachycardia caused by an increased sympathetic tone.However, its specific role in regulating sinus rhythm remains unclear. This study investigates the impact of NOD1 activation on murine sinoatrial (SAN) cells physiology. SAN tissues were exposed for 48hrs to: 1) vehicle, 2) the NOD-1 activator C12-iE-DAP (20 µg/ml), 3) the NOD1 receptor inactive agonist iE-Lys (negative control, 20 µg/ml). Patch-clamp experiments on isolated SAN cells incubated in the three conditions revealed that only C12-iE-DAP increased the spontaneous action potential rate (+26%) and this effect associates with a steeper diastolic depolarization and a larger If-current (~+65%). Importantly, C12-iE-DAP did not affect the ICaL and ICaT currents. Furthermore, C12-iE-DAP reduced β-adrenergic modulation of cell chronotropism both at cellular and at tissue level. In summary, our data suggest the involvement of NOD1 in regulating SAN activity with potential implications in molecular profiling and clinical treatment of pathological cardiac conditions associated with inflammation.

Poster Abstracts

(presenting authors are shown underlined)

P1.1

Identifying Cystatin B interactors in brain cortex development to elucidate molecular mechanisms underlying EPM1 pathogenesis

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Progressive myoclonic epilepsy 1 (EPM1) is a neurodegenerative disease caused by loss-of-function mutations of the Cystatin B (CSTB) gene. Low levels of functional CSTB impact cell differentiation and interneuron recruitment during human brain cortex development. Moreover, CSTB plays a role in synaptic plasticity, indeed it is detected in synapses and released via extracellular vesicles (EVs), as well as in soluble form.
To explore if reduced pathological CSTB levels affect synaptic plasticity in EPM1, we isolated synaptosomes from human cerebral organoids (hCOs) generated from patient cells. Compared to control hCOs, EPM1 hCOs display lower levels of presynaptic proteins and one protein synthesis initiation factor, along with compromised EVs trafficking. Additionally, EPM1 neurons exhibit abnormal morphology, suggesting an altered neuronal connectivity in EPM1.
To investigate CSTB physiological function during neurogenesis and possible differences in cortical areas generating mainly excitatory or inhibitory neurons, we isolated CSTB interactors from dorsally and ventrally patterned hCOs via co-immunoprecipitation (co-IP) analyses. Co-IP was performed at 20 days of maturation of hCOs, when they are composed mainly by progenitors cells, and at 40 days when first neurons arise. Comparing CSTB partners across cortical areas, and at different neurogenesis stages will help to identify physiological pathways that may be altered in EPM1.

P1.2

Ocular dominance plasticity in the adult brain: beyond short-term changes? Insights from 7t bold responses in amblyopic patients

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Short-term monocular deprivation induces transient changes in the adult human visual system, enhancing the representation of the deprived eye. Using ultra-high field 7TfMRI, we showed that these effects are seen in both the primary visual cortex (V1) and the Pulvinar, and also involve a change of Pulvino-cortical functional connectivity at rest. Here we asked whether these short-term effects can be stabilized into long-term changes, beneficial for the treatment of amblyopia in adults.
We performed short-term deprivation of the amblyopic eye (six 2-hour sessions over a 4-week period) while patients (N=8) engaged in physical exercise. We measured V1 responses to monocular visual stimuli, before/after a 2-hour deprivation and at the beginning/end of the 4-week protocol. We found that initial 7T-BOLD responses in V1 were lower for the amblyopic eye than the fellow eye. Fellow eye BOLD dominance decreased after the 2-hour patching and after the 4-week training. Consistently, the amblyopic eye visual acuity improved by 0.10 ± 0.05 logMAR (about 1 line of the 10/10 Snellen chart).
We conclude that short-term monocular deprivation in adult humans induces a plastic change of the visual system with effects that can be stabilized into long-term changes, improving acuity and balancing eye dominance in amblyopes.

P1.3

A cell assembly-based strategy for whole brain interrogation of inter-regional coordination

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Cell assemblies are a long-standing object of interest in neuroscientific research. By being defined as flexible functional groups of neurons with coordinated activity, they have shown to possess coding properties, so that their dynamics of formation and vanishing may reflect cognitive and perceptual processes. These characteristics well suit the definition the cell assemblies earned as the functional units of the brain. In parallel, an evolution in the neurophysiological and analytical tools, which produced cutting-edge algorithms for cell assembly detection, has now made possible to identify inter-neuronal relationships exceeding the pairwise correlation among tens of thousands of recorded cells.
In the present study, we investigate functional relationships between neurons belonging to more than 70 brain regions by looking for the assemblies of 2 and 3 units they are able to form.
2-units assemblies reveal preferential flows of information within a couple of areas, while loop-like assemblies, that is, 3-units assemblies spanning 2 areas, may embody specific motifs of reentrant flow of information.
The brain-wide nature of this study offers the scientific community a database to interrogate and explore in order to identify significant functional interactions, either known or unknown, providing also a tool to answer new questions from a single-cell perspective and at a millisecond timescale.

P1.4

Glycolytic inhibition: an approach toward epigenetic control of neuronal excitability

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Glycolytic inhibition plays a crucial role in the control of neuronal hyperactivity that characterizes seizures. A possible mechanism of action underlying such anti-seizure action has been identified in the powerful epigenetic modulator, REST. We studied the role played by REST on the effects induced by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), on the network activity of cultured hippocampal neurons. 2-DG reduced NADH/NAD+ ratio, increased REST mRNA and protein levels and enhanced its translocation from the cytoplasm to the nucleus. Multielectrode array recording revealed that 2-DG decreased firing and bursting activity of the hippocampal network. GABAergic inhibitory inputs were unaffected, while the amplitude of evoked Excitatory PostSynaptic Currents (EPSCs) were reduced in parallel with a decrease in the amplitude of miniature EPSCs, suggesting a reduced expression of glutamatergic receptors that was confirmed with immuno-blot assays. These results demonstrated that inhibition of glycolysis triggers a REST -dependent scaling-down of glutamatergic inputs through a down-regulation of GluA2 receptors expression at postsynaptic sites. These findings further strengthen the central role of REST in neuronal transcriptional reprofiling aimed at maintaining physiological levels of network activity in front of possible aggression by ictogenic and epileptogenic factors.

P1.5

Acute gut dysbiosis induces alterations in neuronal network excitability and triggers neuroinflammation in the brain

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Inflammatory bowel diseases affect the gastrointestinal tract and can lead to extra-gut effects, including depression and anxiety related to the brain. During the peak of inflammation, certain brain regions, such as the hippocampus, respond to bowel inflammation by modulating neuronal and glial activity. However, a comprehensive understanding of the mechanisms involved remains incomplete. Thus, we developed a mouse model of peripheral inflammation to investigate the impact of the gut-brain axis's impact on brain functioning and identify the mediators of cerebral dysfunctions.
First, we validated our model by characterizing gut inflammation. Our analyses revealed the presence of reactive enteric glia and disruption in gut integrity. Metabolomic analysis confirmed gut dysbiosis, showing alterations in the levels of SCFA. We then explored whether peripheral inflammation induces neuroinflammation in the brain. Studying glial cell responses, we observed changes in morphology and density of astrocytes and microglia. These alterations are associated with changes in hippocampal excitatory and inhibitory synaptic transmission, specifically showing an increased excitability of the neuronal network. In conclusion, so far we have demonstrated that peripheral gut inflammation is mirrored by a neuroinflammatory state in the hippocampus that could potentially disrupt proper synaptic functionality.

P1.6

Modeling the autistic cerebellum: propagation of granule cells alteration through the granular layer microcircuit

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How single neuron alterations propagate through microcircuits remains largely unknown. We addressed this by using a bottom-up modeling approach in a mouse model of autism spectrum disorders (ASD). Cerebellar involvement in ASD is well-documented, e.g. in the IB2-KO mouse ASD model, NMDA receptor-mediated synaptic current and neuronal excitability of cerebellar granule cells (GrCs) are significantly enhanced.
We modified an existing wild-type GrC multi-compartment model to replicate IB2-KO GrC properties, allowing the reconstruction of a granular layer populated with IB2-KO GrCs. Our ultimate goal is to extend this microcircuit to include the molecular layer for a complete cerebellar cortex reconstruction. In the IB2-KO GrC model, we adjusted the tonic glutamate level in the mossy fibers-GrC synaptic model and the NMDA maximum conductance to match the empirical I-f curve and NMDA current changes. This predicts a baseline ambient glutamate level of 28µM and an NMDA conductance change of 7.5 times. Regarding the granular layer, we aim to replicate the previously observed spatially expanded higher E/I balance around IB2-KO GrCs, though it is unclear if altered GrCs or also Golgi cells are involved. For the reconstruction and simulation of the multi-compartment network, we used the Brain Scaffold Builder interfacing with the NEURON simulator. Our model predictions will help clarify the link between microphysiological neuronal changes and cerebellar circuit dysfunction in ASD.

P1.7

The sodium and GABA affinity determines the dual role of Betaine on GABA transport (GAT1)

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Betaine is an endogenous osmolyte with demonstrated neurotherapeutic potential. Recently, we proposed a molecular mechanism for betaine's neuroprotective effects as a GABA modulator of GAT1 (slc6a1). Here, we have examined betaine's action on GAT1 mutants with altered GABA affinity to further explore the relationship between GABA and betaine during the transport cycle.
We have recently shown that betaine helps preserve neurons from excitotoxicity through the temporal inhibition of GAT1. Betaine acts as a slow substrate, occupying the binding site longer than GABA and preventing the fast transition to the inward conformation (IC) that typically happens in the presence of GABA alone. This occurs only when GABA and betaine concentrations are lower than their apparent affinities, which is important for physiological GABA homeostasis.
In GAT1 mutants with altered GABA affinity, in the presence of 100 µM betaine, GABA concentrations required to switch the transporter to IC must be higher than the $K_{0.5}$ for GABA of that specific mutant. Since several mutations are linked to SLC6A1 disorder, a rare neurological condition causing seizures, movement and intellectual disability due to impaired GAT1 function, studying the effects of betaine and GABA on mutant proteins can enhance our understanding of betaine's action in brain conditions and SLC6A1 disorders where GABA homeostasis is disrupted.

P1.8

Does curcumin act as an antioxidant in neural stem cells in health and disease?

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Knowledge of the physiological characteristics of neural stem cells (NSCs) is extremely important to understand the effect of perturbation induced by neurological diseases and to arrange new therapeutical or nutraceutical interventions.
It is well known that spinal muscular atrophy (SMA) is a neurological disease due to gene inactivation that shows strong oxidative stress. Although very effective pharmacological treatments are now available, we hypothesized that an antioxidant supplement could ameliorate the course of the disease, especially for the patients who could not use these drugs.
Frequent dietary consumption has led to the discovery of several compounds' positive benefits. For example, the properties of different antioxidants, such as curcumin, used in eastern nations like China and India, have been known for more than 4,000 years, and it is well recognized that eating a diet high in antioxidants can help people stay healthy, prevent aging and a variety of illnesses, such as diabetes and cancer, and lessen inflammation.
In this work, we studied the changes induced by antioxidant treatment on the physiological characteristics of NSCs produced in healthy and SMA mice. We analyzed the effect of antioxidants, including curcumin, on various physiological features of NSCs and tested the hypothesis of Nrf2-NQO1 pathway involvement. We find confirmation of SMA NSCs physiological improvement; however, this effect was only partially driven by the Nrf2-NQO1 pathway.

P1.9

GDF15-GFRAL signaling drives weight loss and lipid metabolism in mouse model of amyotrophic lateral sclerosis

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Alterations in the central mechanisms that control food consumption are robustly associated with severe symptomatology and poor survival in Amyotrophic Lateral Sclerosis (ALS). Growth differentiation factor 15 (GDF15) regulates body weight under several pathological conditions, including cancer and metabolic disorders, acting through GDNF family receptor alpha-like (GFRAL). However, whether GDF15 plays a role in ALS is unknown. Our study reports that ALS patients and hSOD1^{G93A} mouse model highly express GDF15 in microglial cells – previously shown to regulate feeding behavior; and that hSOD1^{G93A} mice also show higher expression GFRAL in the brainstem. Focal injection of GFRAL-shRNA in the area postrema/nucleus tractus solitarius delays body weight loss, modulating food intake and lipid metabolism. Positive impact of GFRAL blockade was also extended to motor symptoms and survival. Moreover, microglia depletion using PLX5622 decreased GDF15 levels in the brainstem, reducing body weight loss and the expression of lipolysis-associated genes in iWAT. Together, our results show how GDF15-GFRAL signaling plays a key role in ALS progression and symptomatology, and that microglial cells partly regulate this pathway.

P1.10

Action potential-mediated metaplasticity of excitatory synapse LTP

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LTP is a positive feed-back process and may saturate its effects. Hence there is a need for its homeostatic adjustment. One described mechanism is action potential-mediated "metaplasticity": a priming period of postsynaptic action potential activity (pAP-prime) before LTP induction reduces LTP expression. However, experiments designed at testing the effect of pAP-prime have given conflicting results, and a detailed description of metaplasticity is missing. Testing a wide range of pAP-prime frequencies we found that LTP expression was significantly suppressed when induced 2.5 minutes after a 10, 30 or 60Hz pAP-prime, and (not significantly) reduced after a 3 or 90Hz pAP-prime. Conclusions: i) postsynaptic firing activity does exert a homeostatic control of future LTP; ii) this metaplasticity effect is modulated by a wide range of firing frequencies. We speculate that such metaplasticity might be commonly engaged by hippocampal CA1 neurons *in vivo*, with LTP of Schaffer collateral inputs tonically hampered by the firing activity of the same CA1 neurons. [Rat hippocampal slices, evoked EPSPs, CA1 neurons, Schaffer collateral stimulation, pAP-prime: 150 action potentials at various frequencies, LTP: paired theta-burst synaptic stimulation. EPSP amplitude 30min after LTP induction, relative to baseline: ctrl 1.7±0.1, n=13; primed 3Hz 1.2±0.16, n.s, n=9.; 10Hz 0.9± 0.1, $p<0.01$, n=8; 30Hz 1.1±0.08, $p<0.01$, n=13; 60Hz 1.0±0.04, $p<0.01$, n=7; 90Hz 1.4±0.22, n.s., n=9].

P1.11

Voluntary intensive exercise: neuroprotective effects on striatal functions of parkinsonian α-syn mice model

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Parkinson's disease (PD) is a debilitating neurological disorder that strikes 1-2% of the adult population. Although the pathophysiology of PD affects the central, peripheral, and enteric nervous systems, the most characteristic pathology of PD is the loss of the substantia nigra pars compacta dopaminergic neurons.
Over the past two decades, one intervention, voluntary exercise, has been identified that appears to lower the risk of developing PD as well as slow its progression once diagnosed. Epidemiological studies have shown that people who carried out physical activity in their life have a significantly lower incidence of PD.
The overall goal of this study was to examine how early exposure to exercise produces stable changes that underlie long-term neuroprotection in an α-syn nigrostriatal pathology murine model.
Results from electrophysiological recordings reveal that, neurons PFF-injected mice subjected to a 90-day voluntary activity presented a robust corticostriatal long-term-potentiation, lost in the pathological mice model. Voluntary exercise was also able to preserve the physiological spontaneous glutamatergic activity and reduce motor disability in the grip strength and hanging wire tests. These effects are associated with a slower nigrostriatal neurodegeneration in the active animals, indicating a neuroprotection role of voluntary exercise in the early stage of PD.

P1.12

PTBP1 controls self-renewal of human neural stem cells and localizes inside tunneling nanotubes

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We have recently demonstrated that human neural stem cells (hNSCs) communicate with each other through Tunneling Nanotubes (TNTs) exchanging functional organelles through this mechanism. Polypyrimidine tract-binding protein-1 (PTBP1) serves as a master regulator of neural stem cell differentiation. However, it remains unclear whether PTBP1 controls the self-renewal and differentiation of hNSCs and whether TNTs play a role in this context. Here, we found that PTBP1 is highly expressed in hNSC neurospheres and monolayer. RNAi experiments revealed that PTBP1 knockdown significantly reduced the number and size of neurospheres, inhibited hNSCs growth and up-regulates the expression of the neuronal marker β3-tubulin. This strongly suggests a role for PTBP1 in hNSC self-renewal and neuronal differentiation.
Analysis of PTBP1 subcellular localization in hNSC monolayers, conducted using high-resolution confocal microscopy and 3D reconstruction, showed that PTBP1 localizes in the nucleus,as expected, but also within the cytoplasm and inside F-actin and nestin-positive TNTs connecting hNSCs.
These findings highlight the central role of PTBP1 in regulating the self-renewal and differentiation of hNSCs derived from fetal human brain and suggest the intriguing possibility that PTBP1 may be transferred between hNSCs via TNTs. This scenario may indicate a novel role for TNTs in spreading stemness-related signals between stem cells.

P1.13

Functional role of L-DOPA to promote melanogenesis and homeostasis of retinal pigment epithelial cells

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L-3,4-dihydroxyphenylalanine (L-DOPA) is a precursor of melanin synthesis, which occurs in Retinal Pigment Epithelium (RPE) during maturation. On human RPE, L-DOPA acts through its specific receptor, GPR143, by modulating VEGF and PEDF secretion, two key factors for retinal homeostasis. A recent clinical trial highlighted the ability of L-DOPA to mitigate retinal degeneration occurring during Age-Related Macular Degeneration (AMD), the leading cause of blindness worldwide. Here, we aimed to understand the mechanisms of action of L-DOPA on RPE. Firstly, through a bioinformatic analysis, we predicted the major pathways linked to L-DOPA and AMD. The obtained results allowed us to select the two most involved pathways, such as melanogenesis and cellular homeostasis. On this basis, we investigated L-DOPA effects *in vitro* on human RPE cells, under physiological and oxidative stress conditions, which is the main risk factor for AMD. The first evidence related to RPE homeostasis was the improvement of cell viability by L-DOPA treatment. Furthermore, L-DOPA counteracted oxidative stress-induced cell death, through the regulation of VEGF and PEDF levels. L-DOPA supplementation was also able to up-regulate tyrosinase, a key enzyme involved in melanogenesis. These insights provide new understanding of L-DOPA mechanisms of action, necessary for further experimental validation, potentially guiding comprehensive treatment strategies for AMD.

P1.14

Microglia-released extracellular vesicles counteract age-related cognitive impairment and restore microglia homeostasis in aged male and female mice

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Brain aging is characterized by low-grade chronic inflammation and high production of inflammatory compounds. Among the first changes occurring in the brain, there are modifications of microglia, the resident immune cells of the brain, both in morphological and functional phenotypes (i.e., increase of inflammatory markers and dystrophic morphology). These alterations contribute to the age-dependent impairment of neural plasticity, cognitive and behavioral deficits, mainly in the hippocampus and occur differently in aged males and females. Extracellular vesicles (EVs) are key players of the inter-cellular communication between donor and target cells, exchanging a package of information (lipids, proteins and nucleic acids). EVs are emerging as a promising tool to develop revolutionary non-invasive therapies to restore the body homeostasis. We demonstrated that EVs released by healthy microglia, intranasally administrated to male and female late-adult mice (16-18 months) were mainly pynocited by microglial cells into the brain. The treatment reduced the inflammatory profile and recovered the homeostatic morphology of microglia. EVs also reduced anxiety-like behavior and improved synaptic plasticity in the ventral hippocampus. The effects on microglia were dissimilar in male and female mice pointing out sex-differences in the anti-aging effects of EVs treatment. Our findings indicate microglial EVs as an innovative strategy to slow down the effects of aging on brain functioning.

P1.15

Sulfavant A: a small marine-derived sulfolipid aids neuroprotection and reduces memory deficits in Tg2576 mouse model of Alzheimer's disease

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To date, how Alzheimer's disease (AD) progresses, from preclinical to clinical stage, needs to be clarified and this is one of the main reasons why no disease-modifying treatments are available yet. Previously our group demonstrated, in Tg2576 mice, a validated model of AD, an early and selective degeneration of the Ventral Tegmental Area (VTA) dopamine neurons, strictly associated with microglia activation leading to cognitive and non-cognitive deficits. Thus, targeting VTA neuroprotection could slow down the disease progression. In the last years natural compounds gained growing interest in the drug discovery for their neuroprotective properties. Among these, Sulfavant A (SULF A) is a new synthetic marine-derived sulfoglycolipid able to prime the maturation and T cell priming of dendritic cells. Here we tested SULF A as a potential neuroprotective agent on the VTA of Tg2576 mice. After a chronic treatment, 6-month-old Tg2576 mice showed a significant reduction of VTA dopaminergic neurons death, resulting in preserved VTA dopaminergic fibres in the hippocampus. Remarkably, administration of SULF A improves hippocampal memory-related behaviours in Tg2576 mice. Overall, our findings confirm this compound as effective in downscaling neurodegeneration since early AD-like phases.

P1.16

Directional variations in muscle coordination during functional reach

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Clinicians use the Forward and Lateral Functional Reach (FFR, LFR) to evaluate postural stability. Yet, it is unclear whether FFR and LFR measure similar or different aspects of stability. This study aimed to explore muscle coordination patterns in healthy young individuals, providing key insights for future research on elderly.
Seventeen volunteers completed 10 repetitions of one-arm FFR and LFR with their dominant arm. Bilateral activity was recorded by surface electromyography in 16 muscles of trunk, legs, and arms. Muscle synergies were extracted using a non-negative matrix factorization, with the number of synergies identified by the Variance Accounted For. The similarity of synergies’ activation profiles was evaluated using the statistical parametric mapping.
Three muscle synergies were identified for each task. The first synergy was associated with FR execution, the second with braking and returning to an upright posture, and the third with movement stabilisation during arm elevation and lowering phases. As expected, muscle weights differed between the two movements for each synergy, while synergy activations exhibited similar profiles. Despite these similarities, small but significant differences were found in the first and third synergies’ activation profiles. Our findings indicate that, besides sharing some muscle coordination patterns, FFR and LFR exhibit distinct activation strategies possibly referring to different stability strategies.

P1.17

β2-containing neuronal nicotinic receptors (nAChRs) in neocortex development and sleep-related epilepsy

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Autosomal Dominant Sleep-related Hypermotor Epilepsy (ADSHE) can be caused by mutant subunits of heteromeric nAChRs. The 'gain-of-function' $\beta2^{V287L}$ subunit, when expressed in conditional mouse strains up to P15, permanently impairs the glutamatergic input to fast-spiking (FS) parvalbumin (PV)-expressing GABAergic cells, in prefrontal layer V. This suggests that $\beta2^{V287L}$ alters the maturation of glutamatergic synapses on FS neurons, leading to lifelong hyperexcitability because of impaired recurrent inhibition.
Because nAChRs regulate the NRG/ErbB4 pathway, which modulates synaptic maturation of FS neurons, we studied these proteins by immunostaining in brain slices of adult mice carrying or not $\beta2^{V287L}$. An increase of NRG1+ synapses was found in layer V of $\beta2^{V287L}$ mice, along with a selective decrease of the glutamatergic NRG1+ synapses contacting PV+ GABAergic cells. We hypothesize that mutant presynaptic nAChRs may overactivate the NRG/ErbB4 pathway, possibly leading to exhaustion of the powerful glutamatergic input to layer V GABAergic neurons.
The underlying cellular mechanisms are studied by patch-clamp methods in postnatal mouse cortical cultures, up to 14 days *in vitro*. Cholinergic agonists and inhibitors of the NRG/ErbB4 pathway are administered during synaptogenesis. Preliminary results confirm a selective regulation of glutamatergic synapses on FS neurons, which also points to novel molecular targets that could rescue the epileptogenic process during synaptogenesis.

P1.18

Sub-toxic doses of glyphosate impair GABAergic synapses in cultured hippocampal neurons

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Glyphosate (Gly) based herbicides, widely used globally, inhibit the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme expressed in plants but not in mammals. Long-term Gly exposure can cause toxicity in the nervous system, impacting neurotransmission and contributing to oxidative stress, anxiety, and cognitive functions. Although Gly safety studies are ongoing, the mechanistic processes underlying its neurotoxicity remain poorly understood. To address these questions, we investigated the effects of Gly in primary hippocampal neurons focusing on GABergic synaptic function and structure. Using confocal immunofluorescence and patch-clamp recordings, we found that 30-minute Gly administration (3µM) modify GABAergic neurotransmission. In particular we observed a decreased frequency and amplitude of miniature inhibitory postsynaptic currents, indicating the involvement of both pre- and post-synaptic sites. Additionally, Gly reduced the number of release sites, the size of the ready-releasable pool of synaptic vesicles, and the number of postsynaptic $GABA_A$ receptors. Morphological analysis revealed a decreased density of both pre- and post-synaptic inhibitory contacts. In conclusion, our study uncovers previously unknown neuronal and synaptic abnormalities caused by Gly exposure, emphasizing the need for further research into Gly neurotoxicity to understand its complex molecular pathway.

P1.19

Platinum nanozymes counteract photoreceptor degeneration and retina inflammation in a light-damage model of age-related macular degeneration

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Retinal degeneration stems from a harmful cycle of oxidative stress and inflammation caused by genetic defects in Retinitis pigmentosa (RP) or chronic or acute stressors as seen in age-related macular degeneration (AMD). We aim to disrupt this noxious cycle using platinum nanoparticles (PtNPs), which mimic antioxidant enzymes displaying high catalytic activity, biocompatibility, and long-lasting effects.
We performed a single intravitreal injection of PtNPs in two rat models: the Royal College of Surgeon (RCS) rat, a genetic model of RP, and the Light-Damaged (LD) albino rat mimicking AMD.
In the LD model, PtNPs significantly preserved retinal function, evaluated *in-vivo* with electroretinograms. *Ex-vivo* recordings of light-evoked responses from retinal ganglion cells (RGC) using high-density multielectrode arrays showed significant protection of the On-RGC pathway. Morphological analysis demonstrated a preserved photoreceptor layer and reduced inflammation, with lower activation of micro- and macro-glia.
In RCS rats, PtNPs were injected at an early stage of degeneration. This resulted in a significant rescue of visual behaviour, measured by the light/dark box test and the fear conditioning test, that was associated with a significant preservation of the photoreceptors layer, measured by morphological assays.
These results confirm that PtNPs can potentially disrupt the oxidative stress and inflammation cycle, making them a promising therapeutic tool for retinal degeneration.

P1.20

Transcranial Direct Current Stimulation ameliorates cognitive and depressive symptoms in a mouse model of ischemic stroke

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Post-stroke depression is the most common psychiatric disorder affecting almost one-third of stroke survivors resulting in anxiety, hopelessness, anhedonia, together with severe cognitive impairment. Recently, transcranial Direct Current Stimulation (tDCS) has emerged as a promising non-invasive treatment in several neurological and psychiatric conditions. We recently demonstrated that bihemispheric tDCS promoted motor recovery in a mouse model of focal ischemia of the motor cortex obtained by photothrombosis (PMID: 35291824). Here we evaluated possible effects of tDCS on post-stroke cognitive and emotional alterations. Mice received tDCS or sham treatment daily for 3 consecutive days, from 72h post-stroke, and were tested in the novel object recognition, forced swim, elevated plus maze and open field tests 4 weeks after stroke. tDCS mice showed a higher preference for the novel object over the familiar one compared to sham group, indicating reduced cognitive impairment. In addition, tDCS mice showed decreased immobility during forced swim test, suggesting diminished depressive-like symptoms. tDCS treatment also attenuated anxiety-like symptoms, as tDCS animals spent an increased amount of time in the open arms of the elevated plus maze and in the mid zone of the open field compared to sham group. These findings highlight tDCS potential as a therapeutic intervention for post-stroke recovery, emphasizing its role in addressing stroke-related cognitive and emotional sequelae.

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P1.21

Rebalancing Neuronal Activity in 2D and 3D human iPSC based Fragile X disease models modulating the adenosine system

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Fragile X Syndrome (FXS), a leading hereditary cause of Autism Spectrum Disorders, arises from the absence of fragile X mental retardation protein (FMRP), crucial for synaptic plasticity and neuronal growth. While animal models have provided insights, species differences limit their translation to human treatments. Human induced pluripotent stem cells (hiPSCs) from FXS patients offer a novel model for studying the disorder.
This work analyzed neurodevelopmental alteration in FXS using hiPSC-derived 2D and 3D cultures from FXS patients and controls. Our 2D cultures, containing neural progenitors, neurons, and glial cells, showed a persistent neural progenitor population, highlighting FMRP's role in progenitor proliferation and cortical specialization. Further investigation into glutamatergic and GABAergic synaptic development, coupled with calcium activity recordings within neuronal networks, pointed to excessive activity and developmental delays, consonant with recognized FXS attributes. Utilizing this cellular platform, we evaluated the therapeutic efficacy of KW60002, an A2A receptor antagonist previously tested in FMRP-KO mice. Chronic treatment with KW60002 significantly mitigated abnormalities in cortical development and network activity in FXS cultures. These findings confirm the therapeutic promise of KW60002 for FXS and emphasize the importance of humanized models and advanced genetic analysis in developing treatments for neurodevelopmental disorders.

P1.22

The retina – a window on amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a devastating disease caused by degeneration of motor neurons. ALS manifestations become evident when the disease has reached advanced and irreversible stages, thus limiting possible early diagnosis and effective management of the disease. The development of new treatments is limited by a lack of understanding of the biological processes that trigger ALS and promote the neurodegeneration. Neurodegenerative diseases are often paralleled by similar phenomena at the level of the retina. In this study, we analyzed functional and morphological alterations of the retina in a mouse model of ALS carrying a mutation in SOD1, a gene commonly associated with ALS. Longitudinal electroretinographic analysis revealed a time-dependent loss of retinal activity. Retinal dysfunction was correlated with altered retinal layer thickness, as assessed by optical coherence tomography, as well as altered molecular markers of retinal neurodegeneration. Notably, these retinal changes can be detected well-before the typical occurrence of ALS-related motor alterations. The present study provides preliminary insights into the use of the retina as possible benchmark for testing novel pharmacological approaches for the treatment of ALS-related neurodegeneration.

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P1.23

Early suppression of corticospinal excitability during the observation of pictures of hand actions

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The presentation of bodily actions is known to affect the motor system activity in the observer's brain. This motor resonance is thought to assist different motor and even cognitive functions. Nevertheless, the way different indexes of brain motor activity are modulated by presented bodily actions is still debated. Motor evoked potentials (MEPs) are markers of corticospinal drive, and their modulation is better interpreted when baseline activity is available for comparison. Here, we presented participants with pictures of hand actions involving common tools. As control stimuli, scrambled versions of the hand action pics were used. Single pulse TMS was delivered on hand motor cortex at different timings from stimuli onset (150, 350, 500, 700ms) and at the presentation of a baseline (fixation cross) while recording from FDI muscle. Results showed an early (at 150ms) difference in MEP amplitude between hand action and scrambled pics. In addition, at this timing the hand action-related MEPs were significantly smaller than the baseline MEPs, suggesting early motor inhibition. Conversely, at the same timing control stimuli did not differ from baseline. In conclusion, beside a commonly observed corticospinal facilitation, hand action presentation may also rapidly induce corticospinal inhibition. The factors promoting opposite corticospinal modulations still need to be fully elucidated.

P1.24

A Multi-Scale Virtual Mouse Brain for investigating cerebellar-related ataxic alterations

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The cerebellum is key for sensorimotor coordination and its dysfunction causes ataxia, a motor syndrome characterized by deficits in coordination and stability. Ataxia is in several cases correlated with dysfunction and degeneration of Purkinje cells (PCs), the main output of cerebellar cortex. They inhibit the Deep Cerebellar Nuclei (DCN), which then project mainly to brainstem, thalamus, and motor cortex. The impact of PC on brain dynamics and the changes caused by their degeneration are poorly known. This question can now be faced using virtual brain modelling. We first developed virtual mouse brain models employing a high-resolution structural connectivity and neural mass models to represent the dynamics of each brain region. Model parameters were tuned to match rs-fMRI data from mice. We reproduced large-scale effects of PCs degeneration by modifying the local inhibition of cerebellar nodes, leading to an increased hyperexcitability of DCN. However, ataxic alterations occur more realistically on a single-neuron scale. Then, we replaced cerebellar nodes with a spiking neural network of the cerebellar microcircuit. This multi-scale approach allows us to change neural densities, connectivity rules and to heterogeneously modify the properties of single-neuron models in the ataxic cerebellum. Combined with electrophysiological and imaging recordings, these models can be used to investigate the impact of fine-grained neural changes in ataxia on whole-brain dynamics.

P1.25

Duchenne Muscular Dystrophy affects peripheral innervation: observations on the sensory-motor sciatic nerve

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Duchenne muscular dystrophy (DMD) is a neuromuscular disease characterized by the lack of the cytoskeletal protein dystrophin (Dp427). Short dystrophin isoforms such as the Dp116 expressed in Schwann cells (SCs) also exist. Dp116 binds to the dystrophin-associated glycoprotein complex (DGC), crucial for maintaining myelin integrity. Our previous data on the sciatic nerve (SN) of *mdx* mice, an animal model for DMD, revealed significantly lower expression of myelin proteins and GABA receptors, as well as reduced excitability of sensory Aβ-mechanoreceptor fibers, compared to wild-type (*wt*) mice. This study analyzes the levels and localization of Dp116 and three core DGC proteins: α-dystroglycan (DG), β-DG, and β-dystrobrevin. In the SN of *mdx* mice, all proteins exhibit significant reductions compared to *wt* mice, except for Dp116, suggesting that disruption of the complex is a primary alteration. In addition, levels of active matrix metalloproteinases 2 and 9, which target α- and β-DG and may be induced by inflamed muscles, are elevated, potentially affecting the stability of the DGC in SCs. Finally, given that cholinergic signaling is a renowned regulator of axon-SC crosstalk, we analyzed the expression of M2 metabotropic (promyelinating) and α-7 ionotropic (anti-inflammatory) acetylcholine receptors, finding significant reductions of both in *mdx* mice compared to *wt* mice. Collectively, our data suggest that DMD may significantly impact peripheral sensory-motor muscle control.

P1.26

Patients with neurodegenerative diseases show poor reactivity of posterior EEG alpha rhythms during the transition from eyes-closed to -open condition

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In the present study, we tested the hypothesis that the reactivity of posterior resting-state electroencephalographic (rsEEG) alpha (about 8-12 Hz) rhythms during the transition from eyes-closed to -open condition may be abnormal in in patients with dementia due to neurodegenerative diseases as Alzheimer’s disease (ADD), Parkinson’s disease (PDD) and Lewy Body disease (DLB).
A Eurasian database provided clinical-demographic-rsEEG datasets in 35 ADD patients, 65 PDD patients, 30 DLB patients, and 25 matched cognitively unimpaired (Healthy) persons. The eLORETA freeware was used to estimate cortical alpha rsEEG sources.
Results showed substantial (i.e., greater than -10%) reduction (reactivity) in the posterior alpha source activities from the eyes-closed to the eyes-open condition in 88% of the Healthy seniors, 53% of ADD patients, 53% of the DLB patients, and only 37% of the PDD patients. In these alpha-reactive participants, there was lower reactivity in the posterior alpha source activities in the three neurodegenerative (i.e., ADD, PDD, and DLB) groups than in the Healthy group. Furthermore, that reduction of posterior alpha reactivity was stronger in the PDD and DLB groups than in the ADD group.
These results suggest that ADD, PDD, and DLB patients may be characterized by very poor reactivity in the posterior cortical mechanisms desynchronizing rsEEG alpha rhythms in relation to increased vigilance levels as an interesting neurophysiological biomarker.

P1.27

Paclitaxel acutely activates I_{CRAC} in sensory neurons: implications for paclitaxel-induced peripheral neuropathy

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Paclitaxel (PTX) is one of the most widely used antineoplastic drugs in several cancers. The PTX action mechanism involves the binding to the β-tubulin subunits that results in the stabilization of the microtubule polymers and leads to the cell cycle arrest. However, PTX causes various notable adverse events of which peripheral neurotoxicity and neuropathic pain are the most dose-limiting side effects. A number of molecular pathways are affected by PTX in sensory neurons, and among these, alterations in excitability and calcium signalling occur early in response to acute drug application. Here we combined calcium imaging and electrophysiological techniques with a pharmacological approach to characterize in mouse dorsal root ganglion (DRG) neurons the involvement of ORAI channels in the excitability and calcium signals. We found that acute exposure to low dose PTX on DRG neurons activated IP_3-dependent calcium release and the store-operated, calcium release-activated calcium current I_{CRAC}. Lastly, molecular analyses revealed transcriptional upregulation of ORAI1 and STIM2 in a paclitaxel-induced peripheral neuropathy rat model. Our data therefore suggest ORAI and STIM as new molecular targets for the development of therapies against the PTX side effects.

P1.28

Unveiling the potential of PARP1 and Connexin 43 modulation in neuropathic pain models

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Neuropathic pain, a persistent condition affecting millions of people worldwide, represents a major challenge due to its complex pathophysiology and difficulties in developing effective treatments.
In the context on neuroinflammatory-driven diseases, intercellular communication and hyperactivation of poly(ADP-ribose) polymerase (PARP) protein emerged as critical players during the chronicization process.
This study aimed at investigating the efficacy of PARP1 inhibition mediated by olaparib, in alleviating neuropathic pain in a preclinical murine model.
We found that PARP1 inhibition induced a marked reduction in mechanical allodynia and an improvement of motor coordination. Moreover, we observed a significant reduction of astrocytes and microglia reactive activation at the spinal level, coupled with a distinct metabolic signature in olaparib-treated animals.
Given that our findings suggest a mutual influence between PARP1 and connexin 43, a critical mediator of intercellular communication in fueling neuropathic pain chronicization, we performed a proof-of-concept experiment on astroglial Cx43 conditional knockout (cKO) model.
We found that Cx43-based channels are mediating heterocellular coupling between astrocytes and microglial cells and that Cx43-cKO mice showed increased resilience to neuropathic pain induction.
Taken together our findings highlight the therapeuthic potential of targeting PARP1 and Cx43, suggesting new perspectives for neuropathic pain management.

P1.29

A TMS and TMS-EEG study for the investigation of the transcallosal connections of face and hand representation areas in the primary motor cortex

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Conflictual anatomical and neurophysiological data raised the question whether the transcallosal pathway connecting the two representation areas of face primary motor cortex (fM1) is absent or present but poorly active. To answer this question, in the present study a combined transcranial magnetic stimulation (TMS) and electroencephalography (EEG) approach was used to investigate the transcallosal pathway, connecting fM1s and, for comparison, in hand M1 (hM1) from both a spatial and a functional point of view. Eighteen healthy subjects underwent investigation of both hemispheres trough i) a TMS session, to study interhemispheric inhibition (IHI) in both fM1 and hM1 representations, and ii) a TMS-EEG session, to calculate the interhemispheric signal propagation (ISP) for the fM1 and hM1. Results showed the presence of IHI in hM1 but not in fM1 while ISP analysis demonstrated a significant suppression of activity in the non-stimulated hemisphere compared to the stimulated one, regardless the hemisphere and representation area. A significant correlation was also observed between IHI and ISP only when stimulating left hM1. Results from ISP analysis suggest the presence of a transcallosal connection between the two fM1s in humans which is, however, functionally poorly active, as demonstrated by lack of IHI.

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P1.30

Neurodevelopmental and neurodegenerative effects of MAPT IVS 10+16 mutation in 2D and 3D human iPSC-derived cultures

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Tauopathies are key neurodegenerative disorders. Our study explores the frontotemporal dementia MAPT IVS 10+16 mutation using 2D and 3D human iPSC-derived cortical and retinal cultures. We examined effects from neurodevelopment to neurodegeneration with a novel protocol enhancing neuronal differentiation and maturation. Our results show that the tau mutation causes a significant imbalance in 3R and 4R tau isoforms, favoring 4R expression in mutant organoids. This mutation triggers increased tau phosphorylation, pivotal in tauopathy progression.
Moreover, it impacts neuronal integrity and cytoskeleton stability, with fragmented neurite morphology in tau-mutant organoids. Gene expression studies indicated significant transcriptional alterations affecting neuronal differentiation and synaptic maturation. Calcium imaging confirmed impaired network development in tau mutant cultures.
Given the observed downregulation in mitochondrial biogenesis and stress responses, we explored the therapeutic potential of bezafibrate, a PPAR agonist. Bezafibrate treatment normalized mitochondrial function and enhanced neuronal integrity, restoring network functionality in tau-mutant organoids.
Our study highlights the link between neurodevelopmental defects and neurodegeneration, suggesting early interventions in tau dysregulation could mitigate later stages of neurodegenerative diseases, underscoring the utility of human iPSC-derived models in studying complex neurodegenerative diseases.

P1.31

Interleukin-15 alters hippocampal synaptic transmission and impairs episodic memory formation in mice

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Cytokines are potent immunomodulators exerting pleiotropic effects in the central nervous system (CNS). They influence neuronal functions and circuit activities with effects on memory processes and behaviors. In the complex cytokine system, interleukin 15 (IL-15) is considered a bridge between adaptive and innate immune system and it is one of the first upregulated cytokine in neuroinflammation Here, we unravel a neuromodulatory activity of interleukin-15 (IL-15) in mouse brain. Acute exposure of hippocampal slices to IL-15 enhances gamma-aminobutyricacid (GABA) release and reduces glutamatergic currents, while chronic treatment with IL-15 increases the frequency of hippocampal miniature inhibitory synaptic transmission and impairs memory formation in the novel object recognition (NOR) test. Moreover, we describe that serotonin is involved in mediating the hippocampal effects of IL-15, because a selective 5-HT_3A receptor antagonist prevents the effects on inhibitory neurotransmission and ameliorates mice performance in the NOR test. These findings provide new insights into the modulatory activities of cytokines in the CNS, with implications on behavior.

P1.32

Spatial semantics is grounded in visuospatial perception: evidence from tDCS modulation of the right posterior parietal cortex in a lexical decision task in a virtual reality environment

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According to grounded cognition theory, higher cognitive abilities, including language, show a modality-specific interaction with the sensorimotor system. Our work investigates the putative relationship between visuospatial perception and spatial semantics through a lexical decision task. In a virtual reality (VR) environment, participants read meaningful terms among a group of words (near or far space-related) and pseudowords, presented in peripersonal (PPS) or extrapersonal space (EPS). The avatar appeared with arms or handling a tool. The task was replicated after applying cathodal transcranial direct current stimulation (tDCS) on the right posterior parietal cortex (rPPC), involved in PPS processing. Baseline data confirmed our hypotheses of a link between spatial semantics and visuospatial perception: in no-tool condition, Reaction Times (RTs) for space-related words were faster when a term meaning was congruent with presentation distance. Tool use reduced RTs of near space-related words read in far space, remapping EPS as PPS. TDCS had no significant results in no-tool condition. However, we observed a disruption of tool remapping and its effect on near space-related words. These data suggest that interference with visuospatial processing, through a tool or rPPC inhibition, can affect spatial language. Our study shows a close connection between spatial semantics and visuospatial processing, and that it is possible, at least in part, to interfere with this relationship.

P1.33

Influence of lifestyle factors on episodic migraine: focus on dietary habits, physical activity, sleep, and stress management

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Migraine is a highly prevalent and disabling neurological disorder which leads to a high resource and cost burden. Lifestyle management holds the potential to influence the severity of this disease and to recover normal brain functions, with a positive effect on the patient overall health and wellness. In this study, included within the DARE (DigitAl lifelong pRevEntion) project, we analyzed the impact of dietary habits, physical activity, sleep routines, and stress management on the frequency of migraine attacks in adult patients with episodic migraine. Appropriate dietary habits, weight loss in overweight or obese patients, ketogenic diet, and dietary supplements such as vitamins, arise as protective factors against episodic migraine. Physical activity, including yoga training, is able to reduce the frequency of migraine attacks, as well as multidimensional sleep health and psychological intervention based on acceptance and commitment therapy for stress management. In conclusion, this study underscores the importance of tailoring lifestyle modifications in migraine therapy, highlighting the impact of lifestyle determinants as non-pharmacological approaches for chronic disease management.

P1.34

Shifting the focus from vascular to neuronal aspects in diabetic retinopathies to enable alternative treatment strategies such as dietary supplementation with Saffron Repron®

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Diabetic retinopathy is a complication associated with diabetes and is one of the leading causes of legal blindness in adults. The main clinical signs and treatment targets are microaneurysms in the retinal vasculature. Recent research suggests that dysfunction of retinal ganglion cells (RGC) and subsequent apoptosis may occur before the onset of vascular problems.
Our research involved ex vivo light-evoked patch-clamp and high-density multi-electrode array (HD-MEA) recordings from retina explants in a genetic model of diabetes, the Ins2Akita mouse. Importantly, these recordings were conducted before the onset of the vascular signs of retinopathy, providing a unique perspective on the early stages of the disease.
Interestingly, we observed a significant increase in the number of sustained responses to light, with slower dynamics, in Ins2Akita mice compared to the control group. This indicates a notable hyperexcitability of light-evoked RGC responses and alterations in the RGC subpopulations.
This is the first report of early abnormalities in the retina of Ins2Akita mice before the onset of retina degeneration. These findings are crucial in understanding retinopathy's early stages and could lead to early intervention strategies.
Based on these findings, we will test whether Saffron Repron®, a spice that modulates the endocannabinoid system, inhibits metalloproteinase-3 (MMP-3) and the P2X7 ATP receptor, might mitigate/prevent diabetic retinopathy in Ins2Akita mice.

P1.35

Development of 2D and 3D models for studying intercellular communication mediated by tunneling nanotubes in the hypoxic glioma microenvironment

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In the tumor microenvironment, hypoxia controls tumor progression and resistance. Intercellular communication mediated by Tunneling Nanotubes (TNTs) plays a pivotal role in this context. However, the exact role of TNTs in the hypoxic tumor microenvironment remains largely unknown due to the absence of 2D and 3D models useful for analyzing TNT-mediated mechanisms and TNT dynamics.
Here, we developed new 2D and 3D hypoxic homotypic models based on U87 and U118 glioma cells specifically designed to investigate TNT-mediated intercellular transfer of mitochondria and small RNAs in donor/receiver cell co-culture systems.
Donor cells were stained with Mitotrackers or transfected with fluorescent RNA, and F-actin was stained with fluorescent dyes. Receiving cells were stained with DiI dye. Cells were co-cultured in control conditions or in oxygen-glucose-serum deprivation (OGD) in monolayer or in self-assembled 3D tumoroids. Co-cultures were analyzed by live-cell time-lapse microscopy and high-resolution confocal microscopy.
Using these models, we found that OGD induces a significant increase in TNT formation. More interestingly, we observed robust trafficking of mitochondria and RNA mediated by TNTs between glioma cells in both 2D and 3D tumoroid models.
We provide a new model to investigate TNTs in the glioma hypoxic microenvironment and suggest a role for TNTs in the intercellular transfer of organelles and RNA in glioma.

P1.36

REST-dependent homeostatic control of neuronal hyperactivity induced by neuroinflammation

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Neuroinflammation has been implicated in several neurological disorders. Lipopolysaccharide (LPS), a potent immunogenic element, has been used to study the pathogenesis of neuroinflammation. Long-term LPS treatment selectively enhances excitatory transmission in primary cultures of hippocampal neurons contributing to excitatory/inhibitory imbalance. Here, we showed that LPS treatment induces the increased expression of the Repressor Element-1 Silencing Transcription Factor (REST), a transcriptional repressor involved in neuronal excitability and synaptic transmission. Since neuronal hyperactivity induces REST-dependent homeostatic responses that reduce the strength of excitatory synapses, we studied the impact of REST-mediated homeostatic plasticity on LPS-induced hyperactivity. Excitatory postsynaptic currents increased after LPS treatment and were paralleled by a decrease in synaptic facilitation especially when REST was blocked. Cumulative amplitude analysis confirmed that the effects are due to an increase in the probability of release (Pr) that depends on an increase in presynaptic Ca^{2+} through voltage-gated Ca^{2+} channels induced by LPS. Inhibition of REST activity by significantly increased the LPS-induced effects on glutamate release. These results suggest a REST homeostatic action that buffers the increase in excitatory transmission induced by LPS, by downregulating Ca^{2+} influx and controlling the Pr of glutamate release. Funded by PNRR 100008-2022-TF-PNRR-PE_MNESYS.

P1.37

Human bone-marrow mesenchymal stem cell-derived extracellular vesicles, intranasally delivered, rescue motor deficits and promote tissue repair in a mouse model of cortical stroke

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Prompt interventional treatment can significantly reduce stroke-related morbidity and mortality. Among these approaches, stem-cells have emerged as a promising tool to counteract post-stroke neurological alterations.
Here we assessed the efficacy of intranasal delivery of human bone marrow mesenchymal stem cell-derived EVs on mice subjected to focal ischemia of the motor cortex by photothrombosis. Mice received EV treatment consisting of: 0.1×10^9 EVs/dose/day delivered 48 hours after stroke and twice a week for 4 consecutive weeks thereafter. Functional assessments were performed using a battery of tasks to evaluate different motor parameters (i.e, use of preferred paw, coordination, force and fine motor skills) before stroke, 48 hours after ischemic insult and every week up to the end of treatment.
Our results showed that EVs significantly rescued forelimb deficits, corroborated by histopathological analyses showing a reduction of infarct volume and an increase of neuronal survival in the peri-infarct area. Of note, EVs modulated brain immune response, as at the end of treatment, we found reduced glial scar area and thickness and decreased number cells positive for the macrophage/microglia marker Iba-1. One week after stroke, EVs already modulated microglia morphology and increased the level of the anti-inflammatory cytokine IL-10.
In conclusion, our results highlight the translational potential of EVs as a novel tool in post-stroke therapies.

P1.38

FPR-2 as a potential game-changer for treating human malignant glioblastoma

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Glioblastoma (GBM) is an aggressive and malignant brain tumor characterized by rapid growth and invasion of nearby brain tissue, with a median survival of about 15 months. Understanding the underlying molecular mechanisms involved in GBM progression, invasion, and drug resistance is essential for developing effective treatment.
Formyl Peptide Receptor 2 (FPR2) is a G-coupled receptor highly expressed in glioblastoma cell lines and human malignant glioblastoma. Several studies indicate that FPR2 activation can evoke a pro- or anti-inflammatory response depending on the ligands, which may trigger different downstream signaling cascades. In this context, we aimed to study the impact of a new anti-inflammatory synthetic FPR2 agonist, MR-39, on the proliferation and viability of different glioblastoma cell lines. Our findings indicate that MR-39 reduces cell proliferation dose-dependently without affecting cell viability. In particular, it induces G1/S cell cycle arrest by downregulating the cyclin D2 and decreases the G2/M phase transition via the cyclin-dependent kinase 1. Additionally, MR-39 potentially suppresses tumor angiogenesis and migration by modulating genes, such as COX-2 and HIF-1a. These findings suggest that FPR2 activation via MR-39 could provide a promising therapeutic strategy for targeting and controlling the complex pathways involved in the proliferation and migration of glioblastoma, offering a glimmer of hope in the fight against this aggressive disease.

P1.39

Characterization of ERα signaling to cell proliferation induced by chronic and pulsatile E2 stimulation in 2D and 3D cell cultures

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17β-estradiol (E2) plays a vital role in human physiology, binds to estrogen receptors (ERα and ERβ, and its action is determined by the pulsatile secretion in the bloodstream. E2 affects cell proliferation, and dysregulation of E2:ERα signaling contribute to the development of breast cancer (BC). Research on E2:ERα signaling has primarily used 2D cell cultures, which do not fully recapitulate the complexity of tissues and do not consider the E2 pulsatile nature. Thus, we studied E2:ERα signaling in cell proliferation using both 2D and 3D BC cell culture models under continuous and pulsatile stimulation conditions. Results revealed that BC cells grown in an alginate-based 3D matrix exhibited similar responsiveness to E2 compared to cells grown in conventional 2D culture plates. These results support the use of the 3D culture model for studying response to drugs and provide a more realistic microenvironment for such studies. Furthermore, a brief 5-minute exposure to E2 triggered a physiological response comparable to continuous E2 exposure, suggesting that the cellular response to E2 is more important than the continuous presence of the hormone. In conclusion, the alginate-based 3D culture model is suitable for studying the effects of E2, offering a more realistic representation of tumor-microenvironment interactions. The results also highlight the importance of considering the physiological importance of the temporal dynamics in studying E2 signaling and cellular responses.

P1.40

Essential oil from *Origanum vulgare* displays anti-inflammatory and healing activity on human keratinocytes

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The essential oil from *Origanum vulgare* (OEO), an aromatic plant of the Lamiaceae family of the Mediterranean flora, is widely used in folk medicine as a remedy against numerous diseases. The aim of this study was to evaluate the antioxidant/anti-inflammatory/healing capacity of OEO on a human keratinocyte cell line (NCTC 2544), treated with IFN-γ and histamine. OEO characterization was performed by gas chromatography and gas chromatography-mass spectrometry. The viability of the treated and control cells was assessed by the tetrazolium salt metabolization test and crystal violet staining. Antioxidant activity was measured by the determination of reactive oxygen species, superoxide dismutase-1 levels and 1,1-diphenyl-2-picril-hydractil assay. DNA damage was evaluated by 8-hydroxy-2'-deoxyguanosine analysis. The anti-inflammatory capacity of OEO was verified through the measurement of cyclooxygenase-2, intracellular adhesion molecules-1, inducible nitric oxide synthase, and proliferating cell nuclear antigen, by Western blot analysis and Reverse Polymerase Chain Reaction. Extracellular matrix conditions were examined by the evaluation of gene expression and protein synthesis of metalloproteinases-1 and -12. Finally, the "scratch test" was used to evaluate the healing capacity of OEO. This study revealed some mechanisms through which OEO reduces inflammation and promotes cell motility during the healing process.

P1.41

Exploring Cell Mechano-transduction with Dual Laser Optical Tweezers

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Mechanical stimuli from the cell environment are sensed by mechanosensory neurons through stretch-activated ion channels, among which Piezo1 and 2. Piezo2 are highly expressed in dorsal root ganglion neurons (DRGn), where they are located at the periphery of the neurons, mediating touch and proprioception. The polarization is lost in DRGn in culture, in which channels are evenly distributed on the cell surface. The aim of this study is to describe the mechano-transduction causing the opening of Piezo2 and the process leading to their polarization in DRGn. For this, we used dual laser optical tweezers to apply stress to HEK293 cells wild-type (WT, expressing Piezo1), and transfected (T) to overexpress Piezo2. Ramp-and-hold pushes were imposed on the cell membrane to generate stress and indentation, which open Piezo channels and allow Ca^{2+} to flow into the cell. While Piezo2 overexpression in T cells doesn’t affect the mechanical response (defined by the Young's modulus), fluorescence imaging of internal Ca^{2+} shows that: i) fluorescence intensity increases in 27% of WT and 66% of T cells; ii) Ca^{2+} influx for a given indentation depth is fourfold greater in T cells. These results indicate that the fluorescence increase is due to the increase in the number of functional Piezo2 in T cells and validate the method for future studies on Piezo2 response in different cell sub-regions, and on the mechanism of polarization of their expression in DRGn. Supported by MUR-20208TPFLN.

P1.42

Molecular mechanism of Br-SQ-C4 phototoxicity: the role of Ca^{2+} and ROS interplay

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Photodynamic therapy (PDT) is a widely used treatment for both oncologic and non-oncologic diseases. It is based on the administration of a photosensitizer (PS) whose photoactivation induces cellular death due to the interplay of reactive oxygen species (ROS) and Ca^{2+}. Among the different PS, Polymethine dyes (PMD) are promising, due to their biocompatibility, phototoxicity and their absorbance in red-near infrared region of the light spectrum. We characterized Br-SQ-C4 phototoxicity molecular mechanisms to investigate the intracellular signaling using MCF-7 cell line, incubated with the Br-SQ-C4 [1µM] O/N and then irradiated with RED-LEDs (λ=640 nm; Fluency: 3.84 J/cm^2) for 8 minutes. Photoxicity, cytosolic, endoplasmic reticulum (ER) and mitochondrial Ca^{2+} signals, ROS release have been assessed. We demonstrated that Br-SQ-C4 localize at the level of the ER. Following Br-SQ-C4 irradiation $O_2^{-\bullet}$ and $^{\bullet}OH$ ROS are released and intracellular [Ca^{2+}] significantly increases (due to both extracellular Ca^{2+} entry as well as ER Ca^{2+} release) and subsequently uptaken from mitochondria. We demonstrated that ER Ca^{2+} release is a key player in light induced ROS production which in turn regulates ER Ca^{2+} release and the consequent mitochondria Ca^{2+} uptake. Future studies will better identify the role of Ca^{2+} channels in the signal transduction induced by Br-SQ-C4 photoactivation, and their potential role as target to increase the therapeutic efficiency of PS application in PDT.

P1.43

ER stress, honey and keratinocytes

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Keratinocytes are constantly exposed to environmental stressors, which can lead to the accumulation of misfolded or unfolded proteins in the ER. Under ER stress, several signaling pathways are activated to reestablish its homeostasis. However, when cells undergo long periods of ER stress, these signals will eventually turn to be pro-apoptotic. These cellular responses are extensively known and are referred to as UPR (unfolded protein response). UV irradiation and chemicals have been shown in skin cells to induce ER stress and the UPR by altering the protein folding mechanism through ROS-mediated oxidative stress and the efflux of Ca 2+ from the ER. Therefore, antioxidants are able to counteract oxidative stress and can also improve ER functions. It has been already described that several natural antioxidants can modulate ER and its oxidative stress. The most well-known, well-liked, and valued product made by honeybees is honey, which is a complex blend of nutrients and bioactive substances with a variety of biological uses. We administered the ER stress modulator thapsigargin to keratinocytes, and we assessed a
battery of UPR indicators at the gene and protein levels of expression to determine the honey's possible anti-ER stress activity.

P1.44

Effect of Mitochondrial Complex I Inhibitor Rotenone on dopamine release and cell survival

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Rotenone, derived from the roots and stems of Derris, Tephrosia, Lonchocarpus and Mundulea plant species, is considered the classical inhibitor of mitochondrial complex I. However, the mechanism through which it modifies survival functions of neurons remains unclear. In the present study, PC-12 cell cultures were used to investigate its toxic effects on neurotransmitter release and underlying mechanisms of rotenone-induced neuronal cell death. Our results proved how an incubation of 0.5 µM rotenone for 24h on PC12 affects cell ultrastructure, including modifications in mitochondrial structure, a decrease in synaptic vesicles, and the loss of cellular junctions. Furthermore, production and release of ROS and the number of cells undergoing apoptosis are increased. We also observed a reduction on dopamine release caused by impaired mechanisms underlying vesicular exocytosis functions. This study is a common experimental model to investigate the mechanisms underlying the administration of rotenone, a toxic chemical compound used to reproduce dopamine deficiency, one of the most important pathological features of Parkinson's disease. Our results attempt to achieve at new potential therapies for the disease that best take into account the environmental role of rotenone on dopaminergic survival.

P1.45

Cardiotoxicity of proteasome inhibitors in multiple myeloma: differential effects of bortezomib and carfilzomib on calcium dynamics in hiPSC-derived cardiomyocytes

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Bortezomib (BTZ) and carfilzomib (CFZ), proteasome inhibitors (PIs) for treating multiple myeloma (MM), are linked to cardiotoxicity, including arrhythmias, heart failure, and changes in cardiac function. Although CFZ shows higher cardiotoxicity, both drugs cause mitochondrial changes, reduced ATP, and left ventricular dysfunction in animal models. The mechanisms behind these effects remain unclear, possibly involving calcium handling and proteasomal inhibition in cardiomyocytes. Effective cardioprotective measures are still lacking, emphasizing the need for further research.
This study compared the effects of BTZ and CFZ on calcium transients in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Cells were incubated with BTZ (2.8 nM) or CFZ (3.2 nM) for 24 hours. Using Fura-2AM, CFZ was found to reduce transient amplitude (0.32±0.02 vs. control: 0.48±0.02), delay return to baseline (0.63±0.05 ms vs. control: 0.51±0.02 ms), and increase tau (0.83±0.08 vs. control: 0.63±0.02). BTZ only accelerated time to peak (0.36±0.02 ms vs. control: 0.45±0.02 ms).
In conclusion, CFZ and BTZ affect calcium dynamics in hiPSC-CMs via distinct mechanisms, highlighting potential cardiotoxic risks. Further research is needed to clarify these mechanisms and develop strategies to mitigate cardiac adverse effects in MM treatment.

P1.46

Characterization of new class of membrane targeted photo actuators through molecular dynamics simulations

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Modulation of neuronal activity through optical technologies has opened new scenarios for the treatment of neurodisorders like blindness, paralysis and neurological diseases. Azobenzene-based compounds have been used to induce cellular inhibition or excitation following photostimulation. The molecules are based on amphiphilic moieties that dwell in the cell membrane and perturb its structure inducing light-dependent cell hyperpolarization or depolarization.Both steric hindrance, inter-molecular interactions and electronic properties of the actuators are optically modulated. A newly synthetized photoswitch was shown to trigger action potential firing in neurons in response to pulses of visible light. The molecule spontaneously inserts into the plasma membrane and causes its thinning through trans-dimerization in the dark, resulting in an increase in membrane capacitance that is reversed by illumination. New class of opto-actuators with distinct structural features that can either hyperpolarize or depolarize the cells has since been synthetized. Computational approaches like molecular dynamics simulations at atomic or coarse-grained resolution provide a microscopic view of the interactions between the actuators and the lipid bilayer, giving insight on how the compounds permeate the membrane and perturb its structural properties. Here, we present the simulations of various photoswitch-membrane systems and discuss the outcomes within the context of experimental findings.

P1.47

Extracellular acidosis drives pancreatic Vasculogenic Mimicry via Na^+/H^+ exchanger isoform 1 (NHE1) and calcium entry

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Similar to normal stem cells, cancer stem cells (CSCs) exhibits multilineage differentiation potential and plasticity. For example they can express an endothelial-like phenotype and participate in tumor neovascularization by forming a blood-conducting network known as Vasculogenic Mimicry (VM). We previously reported that in pancreatic cancer CSCs develop VM by overexpressing endothelial factors and secreting pro-angiogenic factors. While microenvironmental acidosis is a driver of carcinogenesis especially in the pancreas, its role in VM and the ion transporters involved are unknown. As normal stem cell differentiation is regulated by Na^+/H^+ exchanger 1 (NHE1)-driven pH, we investigated the role of NHE1 and the intracellular signaling involved in the acidosis induced VM using a platform of 3D organotypic cultures on different mixes of Matrigel-Collagen I to simulate malignant progression. Cell ability to form VM was highest on a mix mimicking the normal/early tumor ECM and decreased with increasing Collagen I. In all the ECMs, VM increased with low pHe and both basal and stimulated VM were dependent on NHE1 activity. The stimulation of VM by extracellular acidosis was dependent on the transport of extracellular Ca^{2+} into the cell and the consequent intracellular Ca^{2+} increase. Altogether, these data demonstrate that extracellular acidosis triggers cellular mechanisms that up-regulate VM to overcome the constraints of ECM composition and drive malignant progression.

P1.48

Hypoxia-induced adaptive strategies: involvement of autophagy and sequestosome-1/p62 in human dendritic cells

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Partial oxygen pressure (pO_2), which is strictly dependent on the diffusion gradient between the vascular network and on cellular O_2 consumption rates, is frequently low within peripheral tissues, which are, therefore, characterized by hypoxia.
This condition clearly affects the physiology of dendritic cells (DCs) which are distributed in various body districts, in both lymphoid and non-lymphoid tissues, which are characterized by different pO_2 gradients. In this context, they continually capture self-antigens in order to spread tolerance, contributing to maintenance of immune homeostasis.
Autophagy has been demonstrated to act as a hypoxia-induced adaptive strategy in several cell types, accelerating the degradation of the cargo receptor Sequestosome-1/p62 (p62), which, interestingly, is associated to hypoxia-inducible factor (HIF)-1α stabilization.
We here investigated the impact of the autophagic protein p62 in human monocyte-derived DCs exposed to hypoxia (2% O_2), by either using an autophagy inhibitor (SAR405), which was able to impair p62 degradation, or inhibiting p62 through an RNA interference approach. By the combination of immunofluorescence confocal analysis, Western blotting and RT-qPCR, we showed that p62 is crucial to maintain DC viability under hypoxia. Indeed, it is essential not only in ensuring selective autophagy but also in the promotion of pro-survival responses, mainly through MAPK modulation.

P1.49

Molecular and cellular pathways contributing to brain aging: focus on isothiazolinone

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A significant number of emerging pollutants resulting from point and diffuse pollution is present in the environment. These are chemicals that are not commonly monitored but have the potential to enter the environment and cause adverse ecological and human health effects. Recent evidence demonstrated that a new family of organic pollutants released directly or indirectly into the environmental through wastewater was represented by isothiazolinone family. Doing a search on Pubmed, we find few results on these compounds, even less on any toxic effects that they could have and unfortunately even less on their mechanism of action. Until today some authors demonstrated that after the exposure there was the release the upregulation of kinases (MAPK) signaling pathway, and as a consequences with cell death, and neurodegeneration. With this background in our mind we evaluated for the first time whether exposure to CMIT/MIT induced cell death and brain aging and how it did so. In our study, we have shown that CMIT/MIT exposure inhibits SHSY5Y cell proliferation and induces apoptosis associated with up-regulation of MAPK pathway. Therefore, these findings suggest that CMIT/MIT from consumer products might be one of public health threatening-risk factor in various diseases.

P1.50

Envisioning the structural foundations for sodium channel Nav1.2 modulation by PRRT2

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Proline rich transmembrane protein 2 (PRRT2) has been categorized as the unique causal gene for a wide spectrum of paroxysmal movement disorders (PMD). It is expressed at the synaptic compartment where it actively contributes to membrane intrinsic excitability regulation. PRRT2 has been proven to associate with the neuronal voltage-gated sodium channel Nav1.2, causing a reduction in channel's membrane exposure as well as a negative shift in its voltage dependence of activation and a delay in its recovery from the inactive state.
Even though the Nav-PRRT2 interaction has been confirmed experimentally, no detailed structural information about the complex is currently available. To fill this gap, we designed a multi-scale computational workflow to generate and rank a set of putative protein-protein contact interfaces, to be submitted to experimental validation. The procedure includes extensive atomistic molecular dynamics (MD) simulations to generate broad ensembles of PRRT2 transmembrane domain (TMD; G261-K340) and Nav1.2 conformations, protein-protein docking and coarse-grained MD (CG-MD) simulations. Results provided multiple PRRT2-Nav1.2 complexes that were analyzed to identify key interface amino acids to be subjected to mutations and experimental functional studies.

P1.51

Mitochondrial function in Amyotrophic Lateral Sclerosis caused by the pG376D TARDBP Mutation

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Many diseases have been linked to mitochondrial dysfunctions and several studies suggest that these dysfunctions play a role in Amyotrophic Lateral Sclerosis (ALS). Different mutations have been reported to cause ALS, a neurodegenerative disorder. A few years ago, a novel rare pG376D mutation in TARDBP gene, encoding the TAR-DNA binding protein 43, was reported in ALS. This study aims to characterize mitochondrial function in models of ALS-associated TDP-43 pG376D mutants that could underlie axonal degeneration found in patients. The experiments were performed on HEK293T and Neuro2a cells transfected with plasmids to induce the overexpression of TDP-43 wild-type or carrying the mutation. Moreover, patient fibroblasts carrying the ALS-associated p.G376D mutation at the onset were used. The results showed an alteration in mitochondrial functionality both in wtTDP43 and mutTDP43 cells when compared to the control. Particularly, in both cell lines we found a decrease in mitochondrial membrane potential, cellular respiration, and Cytochrome C Oxidase activity. In mutTDP43 cells we also observed an increase in oxidative stress and an impairment of antioxidant machinery. In addition, we observed a strong reduction in cellular respiration in patient fibroblasts carrying pG376D mutation at the onset. These results increase our understanding on molecular mechanisms underlying ALS pathogenesis associated with TDP-43 pG376D mutation and could help to identify new therapeutic strategies.

P1.52

P2X7 receptor activation suppresses cell migration and induces membrane hyperpolarization in kidney renal clear cell carcinoma

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P2X7 receptor is an ATP-gated plasma membrane ion channel that plays a crucial role during oncogenic transformation in various malignancies. In colorectal cancer, P2X7 stimulates tumorigenesis. It also promotes growth and migration of breast tumor cells. While purinergic receptors are abundant in urological cancers, P2X7's role in kidney renal clear cell carcinoma (RCC) remains largely unknown. This study explores the function of P2X7 in 786-O cells, a human RCC cell line. We showed that these cells express P2X7, predominantly localized in the cytosolic compartment. Ca2+ signaling experiments using Fura-2 demonstrated that acute 100 µM ATP-induced Ca2+ responses were entirely dependent on extracellular Ca2+, implicating an ionotropic response. Interestingly, 24 hours ATP stimulation (100 µM) significantly reduced cell migration (migration percentage at 24h in ctr: 95.44% ± 1.8 and ATP-stimulated cells: 79.75% ± 4.3; p-value ≤ 0.005, t-test. Data expressed as mean ± SEM) without cytotoxic effects. Finally, while 786-O cells possess depolarized membrane potential (Vm)(-15.57mV±2), chronic 100 µM ATP stimulation significantly hyperpolarized their Vm (-23.40mV ± 2.27; Data given as mean ± SEM; ctrl vs ATP stimulation p-value ≤ 0.05, t-test). Given that the fluctuation of Vm can functionally regulate tumorigenesis, dissecting the effect of P2X7 receptor activation on cancer dissemination will open a promising avenue in the fight against RCC.

P1.53

Astrocyte-derived small extracellular vesicles hinder glioblastoma malignancy

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Glioblastoma (GBM) is the most common and deadly brain tumor, with a poor prognosis due to high rates of recurrence. This recurrence happens because GBM cells invade surrounding healthy brain tissue, creating new tumors. A crucial aspect of this invasion is the GBM cells' ability to regulate their volume and shape, primarily through ion channels like the volume-regulated anion channel (VRAC) and the Ca2+-activated K+ channel (IK).
The peritumoral cellular microenvironment, which includes a mix of tumor and non-tumor cells such as astrocytes, plays a significant role in GBM progression. Extracellular vesicles (EVs) facilitate communication between GBM cells and astrocytes, influencing whether astrocytes support or inhibit tumor growth depending on the tumor's stage.
Recent research focused on small EVs from healthy (antitumoral) astrocytes (ADEVs) and their role in combating GBM. These ADEVs contain miR124, a molecule that impedes tumor growth in mice. miR124 in ADEVs reduces GBM cell volume regulation and migration by decreasing the activity of VRAC and IK channels. These findings suggest that ADEVs/miR124 could be a promising therapeutic approach for GBM treatment.

P1.54

Protective effects of plant-derived matrices extracts against fatty acids-induced hepatic steatosis in Hepg2 cells

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Plant-derived matrices may represent a valuable source of bioactive compounds. Several studies highlighted their properties to provide new insights into the development of functional foods against obesity-associated diseases. This project aimed to investigate how matrices extracts, such as Tropea red onion peel (T), Montoro coppery onion peel (M), Calabrian *Glycyrrhiza glabra* leaves (L) and *Ceratonia siliqua* leaves (C) can play their possible therapeutic effects in lipid-induced hepatic steatosis. The experimental design was based on the analysis of the dose-dependent effects of these extracts at 6.25, 12.5, 25, 50 µg/mL doses, combined with 500 µM saturated fatty acid palmitate (PA), on cell viability (MTT assay) and lipid accumulation (oil red staining) in an *in vitro* cellular hepatic model (Hepg2). PA induced a marked lipid accumulation into cells, in a time-dependent manner. In fact, the amount of lipid droplets is higher after 48h than after 24h treatment. This effect was attenuated by the combination with the highest doses of these matrices' extracts. PA also induced a significant decrease in cell viability, while the matrices extracts did not induce any variations. In conclusion, these preliminary results showed that the plant matrices extracts used in the present study could be able to counteract PA-induced hepatic lipid accumulation. Further studies are needed to better understand the cellular mechanisms involved into this protective effect.

P1.55

β3-adrenoceptor role in hypoxia-driven retinal neovascularization: insights from the mouse model of oxygen-induced retinopathy

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In the retina, β-adrenoceptors (ARs) play a role in regulating vascular responses to hypoxia. While the involvement of β1/2-ARs in promoting hypoxia-driven neovascularization (NV) is well established, the role of β3-AR remains to be determined. Since β3-AR expression in the retina is dependent on oxygen level, we explored the role of β3-AR in hypoxia-driven NV using a mouse model of oxygen-induced retinopathy. Mouse pups were exposed to 75% oxygen from postnatal day (PD)7 to PD12 (hyperoxic phase), to then return to room air until PD17 (hypoxic phase). At PD17, the retina displayed vascular abnormalities including central vasobliteration and mid-peripheral NV as the result of unbalanced pro- and anti-angiogenic factors. These abnormalities correlated with dysfunctional electroretinogram possibly due to altered activity/viability of retinal neurons, including retinal ganglion cells. β3-AR agonism with BRL37344, given subcutaneously during the hypoxic phase, rebalanced the levels of pro- and anti-angiogenic factors thus counteracting pathological NV. Moreover, BRL37344 promoted central retinal revascularization likely through the recovery of astrocyte template, thus ultimately improving retinal ganglion cell activity and viability. Our study reveals that β3-AR exerts a functional role in the retina. In particular, the finding that β3-AR may serve as a crucial intermediary in hypoxia-dependent NV suggests it as a possible target for the treatment of proliferative retinopathies.

P1.56

Combination of metformin and cinacalcet slows cystogenesis in human cysts obtained from an ADPKD patient through activation of the AMP-activated protein kinase (AMPK)

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by mutations in *PKD1* or *PKD2* genes and is characterized by excessive cell proliferation and fluid secretion, resulting in cyst formation and growth. Activation of AMPK has emerged as promising therapeutic strategy to slow renal cystogenesis in ADPKD. Metformin, a drug in wide clinical use, is a pharmacological activator of AMPK, whose activity is inappropriately suppressed in ADPKD. We have previously showed, both *in vitro* and *in vivo*, that activation of the Calcium Sensing Receptor (CaSR) with calcimimetics, restores normal levels of AMPK as well as the principal cellular ADPKD dysfunctions, along with significant decrease in cystic volume index. Here, we demonstrate the efficacy of both metformin (200μM for 72h) or the calcimimetic cinacalcet (1nM for 72h) in reducing the increase in volume of human cysts obtained from an ADPKD patient, in response to the cAMP-elevating agent forskolin (6.7% n=29, and 17.7% n=28, respectively). Of note, when cysts were exposed to both metformin and cinacalcet, the effect of the combined treatment in reducing cysts volume was significantly stronger than the mono treatment (29.27% n=28, $P<0.0001$). Together these data indicate that the combination of these two drugs already used in patients, targeting altered AMPK and calcium signals in ADPKD, appears to have an additive beneficial effect, rendering the proposed study promising to therapeutic investigation.

P1.57

Highlighting the relevance of Carbonic Anhydrases in cell migration under hypoxia

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Carbonic anhydrases (CAs) are metalloenzymes, which play a pivotal role in regulating intracellular pH either in physiological and pathological cellular processes. Recently, it has been shown that specific CAs, such as CAIX, are induced by hypoxia and regulate cell survival and migration in several cell types. Among these metalloenzymes, a novel role for CAXII is emerging. For these reasons, we decided to investigate whether hypoxia may affect CAXII expression and its role in cell migration. To this end, we exposed to hypoxia either human melanoma cell line and primary monocyte-derived dendritic cells (DCs), which are characterized by an effective cell migration phenotype. Our results indicate that hypoxia enhances CAXII expression in either melanoma cell lines and primary DCs, along with increased cell migration. When CAXII was downregulated by siRNA, cell migration was significantly impaired. These results were confirmed by using a CAs pan-inhibitor, acetazolamide. In addition, we extended our investigation on the impact of hypoxia on small extracellular vesicles (sEVs) release. Interestingly, hypoxic melanoma cells release sEVs, expressing CAIX mRNA, which was confirmed at protein levels. The overall results highlight the relevance of CAs not only as pH regulators, but also in other cellular processes, such as cell migration, especially under hypoxia.

P1.58

Metabolic reprogramming and Hedgehog pathway in myeloid lineages under hypoxic conditions

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The connections between Hedgehog (Hh) signaling and metabolism are multiple. The Hh pathway is active during embryogenesis and regulates morphogenesis, proliferation, and differentiation. In adult life it is quiescent, but its physiological reactivation occurs in stem cell compartments as a response to tissue damage and drives metabolic reprogramming. It has also been known that a high glycolytic metabolism distinguishes tumors from normal cells. We decided to investigate the metabolic changes induced by Hh pathway modifications in models of chronic myeloid leukemia (CML). CML is a myeloproliferative disorder of hematopoietic stem cells that originate and proliferate in the bone marrow, a hypoxic microenvironment with an oxygen concentration ranging from 6% to almost anoxia. In our work, by using pharmacological approaches, we inhibited or stimulated the Hh pathway, in both aerobic and hypoxic conditions and we evaluated the potential proliferation and metabolic switch. Particularly, we found that the protein expression of some critical transporters and enzymes involved in glycolysis was modified. In addition, we determined the variation in glucose uptake, lactate and ATP production. Interestingly, all these changes seemed to be related to the activation of AMPK, a recently discovered pathway modulated by Hh signaling. These results suggest novel potential therapeutic strategies taking into consideration the hypoxic microenvironment where myeloid lineages develop.

P1.59

Voltage dependent potassium currents in human granulosa cells from follicular fluid of IVF of poor and normal responder patients

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Granulosa cells (GCs) support the development of the oocyte from its earliest primordial follicle stage, playing an essential role in the oocyte maturation and human reproduction, providing energy, and steroidogenesis. Voltage-gated potassium channels are the principal determinants of membrane potential in a variety of cell types in living organisms, however their functional role in the GCs and reproductive disorders is less understood. Follicular GCs were obtained after oocyte retrieval from twenty patients undergoing in vitro fertilization (IVF) during assisted reproductive techniques and analysed by patch clamp technique in whole-cell dialyzed configuration. In this study, we have characterized three different types of potassium currents: i) a large-conductance TEA sensible calcium-activated potassium current (BKCa), ii) voltage-activated outward with rapid activation and inactivation (IA) and iii) a PAP-1 sensible rapid activation no inactivation (DRK) currents, Different expression and biophysical properties on voltage dependent potassium currents are observed between granulosa cells obtained from follicular fluid of poor- and normo- responder patients based on Bologna criteria. These differences could be explained the reduced responses of granulose cells to gonadotropins promoting steroidogenesis in poor responder patients.

P1.60

Physiological responses of early developmental stages of *Mytilus galloprovincialis* to ocean warming and pathogen infection in a global change scenario

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Marine bivalves inhabiting coastal areas are subject to fluctuations of various environmental parameters and evolved physiological adaptation mechanisms to deal with a variety of stressors, including hypoxia, increased temperature, bacterial challenge exposure to pollutants. However, in a global change scenario, these stressors can be exacerbated and occur simultaneously, posing a risk to the health and survival of marine species, in particular of more sensitive larval stages. In this work, responses of early larvae (24 and 48 hpf) of the mussel *Mytilus galloprovincialis* to ocean warming and pathogen infection, alone and in combination, were evaluated. Normal development and survival were evaluated at 48 hpf, and gene expression at both 24 and 48 hpf. Increases in T alone (18>20>22°C) induced alterations of larval shell phenotypes and affected the time course of basal gene expression across development. Combined exposure to increasing T and concentrations of the emerging pathogen *Vibrio coralliilyticu*s resulted in higher larval pathogenicity at 48 hpf, with interactive effects. In conditions that did not affect larval survival (T: 18-20°C; *Vc*: 10^4 CFU/mL), gene transcription revealed both common and distinct targets for the two stressors, with significant interaction at both 24 and 48 hpf. Even if temperature is the main environmental driver affecting mussel development, sea warming will increase the susceptibility to *vibrio* infection, with consequences on mussel populations.

P1.61

Exploring the functional effects of the ACE inhibitor Enalapril and its metabolite Enalaprilat on *Mytilus galloprovincialis*

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Pharmaceuticals in water environments are posing a risk to the whole ecosystem, challenging animal limits for adaptation.
The antihypertensive Enalapril (EN) and its metabolite Enalaprilat (ENT) are found in wastewater and urban effluents. However, data on their effects on the health of aquatic animals are limited.
This study analyzed the effect of EN or ENT on functional parameters of *Mytilus galloprovincialis*, a sentinel of water pollution. Mussels were exposed for 10 days to 2 concentrations of EN [0.6 ng/L (E1); 600 ng/L (E2)], or ENT [7ng/L (ET1); 7µg/L (ET2)] to analyse: cell viability [digestive gland (DG) and hemocytes], cell volume regulation (DG cells), and oxidative stress markers (DG and gills).
At the highest concentrations, both drugs compromised DG cell volume regulation, but did not affect DG cells and hemocytes viability. In the DG, EN unaffected oxidative status, while ENT increased catalase activity. In the gills, EN reduced superoxide dismutase activity in E1 group, and modulated lipid peroxidation and protein carbonylation, while ENT increased catalase activity and affected lipid peroxidation. A modulation of HSPs expression was also observed.
Our results showed that, in *M. galloprovincialis,* EN and ENT elicited a tissue-specific modulation of oxidative status and compromised DG cells ability to face osmotic changes, with potential consequences on animal performance.

P1.62

Understanding benzisothiazolinone's impact on aquatic organisms: insights into physiological and cellular responses

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The widespread use of Benzisothiazolinone (BIT) as a biocide in construction materials and cleaning agents contrasts sharply with the limited understanding of its effects on aquatic ecosystems. This study delves into the physiological and cellular responses of *Mytilus galloprovincialis* cells when exposed to BIT. Experimentation involved exposing cells from both haemolymph and digestive glands to two distinct BIT concentrations (0.03 µg/mL and 0.3 µg/mL) for different times (1 hour, 3 hours, and 24 hours). Assessment of cell viability employed Trypan blue exclusion and Neutral Red retention assays, alongside scrutiny of phagocytosis ability. The investigation extended to evaluating gene expression pertinent to oxidative stress via qPCR. The research outcomes revealed a spectrum of physiological, cellular, and gene expression alterations consequent to BIT exposure. Notably, BIT exposure affected lysosomal membrane stability, phagocytosis activity, and impaired the antioxidant cellular system in aquatic organisms. This comprehensive analysis underscores the implications of BIT exposure on *M. galloprovincialis* and advocates for the exploration and development of environmentally sustainable alternatives to mitigate potential harm.

P1.63

Physiological response to seasonal variation in gills and digestive gland of *Mytilus galloprovincialis*

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Bivalves as *Mytilus galloprovincialis* are suitable organisms for monitoring aquatic environments providing accurate and reliable biological endpoints for evaluating environmental quality.
This is a preliminary study aimed to analyse the influence of seasonal variability, and of anthropic impact, on the physiological status of the mussel *M. galloprovincialis* transplanted to the coastal waters of the North Ionian coast of Calabria. Evaluations were based on biomarkers of oxidative stress and expression of stress proteins. The activity of Superoxide Dismutase (SOD), Catalase (CAT), and the levels of oxidized lipids and proteins (TBARS and OMP, respectively) were analysed in two target organs: gills and digestive gland (DG). HSP70 expression was also evaluated.
Tissue- and season-dependent responses were observed: in the DG, significant variations in SOD and CAT activity were observed particularly in spring, summer and autumn, while in the gills, enzymes were modulated in winter and spring. Similarly, lipid and protein oxidative status was influenced by the season. No significant changes were detected in HSP70 expression. Season-dependent effects revealed both similarities and differences with those reported for other Mediterranean mussel populations. Analyses are in progress with the purpose to correlate the observed changes with environmental stressors, including those imposed by human-derived activities.

P1.64

Exploring taste sensitivity in varied dietary patterns

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The influence of food choice on human health is complex, with taste perception and preferences playing a crucial role in shaping dietary habits. Currently, there are various modern diets that include or exclude different foods and whole food groups, which can affect taste perception and food selection. The aim of this study was therefore to understand how dietary patterns, excluding whole food groups from the diet relate to taste sensitivity. A total of 82 subjects were categorized into four groups: Omnivore (n=22), Vegan (n=29), Vegetarian (VE) (n=17) and LCHF (n=14). For all participants, detection thresholds (DT) for sweet, salty, bitter and umami tastes were determined based on the three-alternative forced-choice method. There were differences between dietary patterns and nutrient intakes and biochemical values ($p < 0.05$), but no significant differences in DTs for tastes between the dietary groups. Individuals who did not perceive one or more tastes were defined as hypo-tasters (HT) and 41.2 % of VE were included in this group. The HT group tended to have lower energy, macro- and micronutrient intakes than tasters, but significant differences between HT and taster groups were observed only in intakes of vitamin B_{12} ($p=0.037$) and zinc ($p=0.041$). Additionally, a negative correlation between DT for salty and fiber intake ($r = -0.549$, $p=0.012$) was observed in the HTs group. Excluding whole food groups can affect biochemical parameters and taste sensitivity.

P1.65

Motility of *Mytilus galloprovincialis* hemocytes: a novel approach for detecting cellular responses to the exposure to emerging pollutants

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Cell motility is a fundamental cellular function involved in several physiological processes. Despite its basic role in physiology, it has been poorly investigated concerning its sensitivity and response to pollutant exposure.
This work aimed to characterize the spontaneous motility of the circulating immunocompetent cells of bivalve mollusks, hemocytes, and to explore *in vitro* the sensitivity of this cellular function to emerging pollutant exposure focusing on one of the most diffused pharmaceutical released into the environment, paracetamol. The study was carried out on *Mytilus galloprovincialis,* a widely recognized sentinel species for marine pollution.
Hemocytes plated in a TC-treated polystyrene 96-well microplate, analyzed by time-lapse microscopy and cell tracking, revealed a spontaneous cell movement with continuous shape changes and pseudopods formation. p53, ROCK, and MEK kinase signaling pathways were involved in the hemocyte motility regulation as assessed by the use of specific inhibitors.
The *in vitro* sensitivity of hemocyte motility to paracetamol exposure was investigated after 1h and 24h exposure. The drug induced alterations in the hemocyte motility behavior with cell-type specific effects on velocimetric parameters.
In conclusion, the study of hemocyte motility offers new sensitive tools for assessing the impacts of emerging pollutants at cellular levels in non-target organisms opening new perspectives for the development of novel biomarkers and/or bioassays.

P1.66

Goldfish redox homeostasis adaptation to hypoxia

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Animals living in freshwater experience continuous fluctuations in oxygen availability due to natural or anthropogenic causes and have developed survival mechanisms implying the onset of a hypometabolic state. The use of O_2 by aerobic organisms is closely linked to the production of reactive oxygen species (ROS) which can act both as harmful and signalling molecules and whose harmful action is finely controlled by the antioxidant system. Goldfish are champion of hypoxia tolerance, but how hypoxia affects their ROS production is currently unknown. Furthermore, little information is available so far on the effects of emerging pollutants, such as some life-saving drugs, whose concentrations in the environment are continuously increasing. We evaluated the effects of 4 and 20 days of hypoxia on parameters related to oxidative metabolism (oxygen consumption and cytochrome oxidase activity) and ROS production (total ROS content, markers of oxidative damage to lipids and proteins, susceptibility to oxidants, total antioxidant capacity). Our data show that during hypoxia, decreased metabolic capacities are associated with a reduced ROS content which, even if not accompanied by reduced oxidative damage markers content, is accompanied by a reduced susceptibility to oxidants, which agrees with the increase in total antioxidant capacity. This is a preliminary study in which the evaluation of the effects of some emerging pollutants on adaptations in animals tolerant to hypoxia will follow.

P1.67

The “ELPME” project: *in vitro, ex vivo* and *in vivo* studies for the evaluation of the effects of low dose exposure to mixture of pollutants in the onset of inflammatory bowel disease (IBD)

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Humans are daily exposed to a wide range of environmental pollutants, which may compromise the homeostasis of organs and tissues by inducing inflammation and oxidative stress, thereby constituting a risk factor in the onset of Non-Communicable Diseases (NCDs), such as Inflammatory Bowel Disease (IBD).
In this regard, the “ELPME” project (PRIN 2022- Next Generation EU) aims to evaluate the effect of low dose exposure of environmental emerging contaminant mixtures, such as the 2,2'4,4'-tetrabromodiphenyl ether (BDE-47) and Estrone (E1), in concentrations similar to real life conditions, by means of *in vitro* (murine RAW 264.7), *ex vivo* (human PBMCs) and *in vivo* (DNBS-Induced Colitis Rats) model systems.
At first, the immunotoxic and immunomodulatory effects induced by single or mixtures of pollutants were evaluated in the RAW 264.7 cell line using a range of concentrations of BDE-47 (3µM-3nM), E1 (100nM-0,1nM) and mixtures at different concentrations and time points. This initial set of *in vitro* data show that “real life” concentrations of the single pollutants do not affect cell viability (MTS assay) and that one of the tested mixtures (30nM BDE-47 + 0,1nM E1) slightly reduces the cell viability (about 20%) at 72h. No differences in ROS production have been observed. Real time analyses suggest that different mixture concentrations are able to modulate IL-6 and IL-10 expression.
Additional data will be collected using RNA seq to analyse differentially expressed genes.

P2.1

Dopaminergic neurons derived from hiPSCs in multiple system atrophy exhibit distinct morphological and functional characteristics compared to healthy controls

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Multiple system atrophy with predominant parkinsonism (MSA-P) is a sporadic, adult-onset, and fatal neurodegenerative disease characterized by severe clinical progression. The pathology of MSA-P involves abnormal misfolded α-synuclein inclusions in brain neurons and oligodendroglia, leading to cell death and a reduction of mesencephalic dopaminergic (DA) neurons.
The study aims to investigate the morphological and electrophysiological features of DA neurons derived from induced pluripotent stem cells (iPSCs) at 35-45 days of differentiation, obtained both from MSA-P patients and healthy control.
The morphological comparison between patients and controls DA neurons showed a significant reduction of dendritic length ($F(4, 245) = 33.10$, $P<0,0001$), number of primary dendrites ($F(4,395) = 11.44$, $P<0,0001$) and soma area ($F(4,395) = 59.33$, $P<0,0001$).
Patch-clamp findings revealed notable distinctions in DA neurons from MSA patients compared to controls, highlighting a reduced cell capacitance (24,36±7 pF vs 40,01±10 pF, $P< 0,0001$), a comparable resting membrane potential (-48,6±6 mV vs -51.9±6 mV, $P= 0.02$), and higher membrane resistance (463.9±220 MW vs 256.4±97, $P<0.0001$ MW). Notably, the rheobase current was markedly lower in MSA patient-derived cells (15,7±7 pA) compared to controls (23±13 pA, $P=0.002$).
Our findings suggest that DA neurons in MSA-P exhibit impaired morphology alongside changes in neuronal excitability and responsivity.

P2.2

Spontaneous GABAergic synaptic currents are modulated by α4β2* nicotinic acetylcholine receptors in human epileptic brain

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In the temporal cortex of patients with temporal lobe epilepsy (TLE) the γ-aminobutyric acid type A receptor ($GABA_AR$)-mediated currents are functionally impaired. Given that the heteromeric nicotinic acetylcholine receptors (nAChRs) containing the α4 and β2 subunits (α4β2* nAChRs) promote neurotransmitter release in several regions of the brain, we investigated the ability of α4β2* nAChRs to enhance the $GABA_AR$-mediated synaptic transmission in human epileptic cortical tissue of surgical origin. We recorded spontaneous postsynaptic inhibitory $GABA_AR$-mediated currents from L5 pyramidal neurons in the TLE cortex, before and during selective administration of desformylflustrabromine (dFBr, a selective α4β2* nAChR positive allosteric modulator, PAM). We observed an increase in spontaneous inhibitory post-synaptic currents frequency (sIPSCs), blocked by dihydro-β-erythroidine (dHβE), a selective α4* antagonist. α4β2* nAChRs activation enhanced the excitability of interneurons (but not of L5 pyramidal neurons) by lowering the action potential threshold. Furthermore, α4β2* activation did not alter inhibitory miniature currents (mIPSCs) recorded from pyramidal neurons. These findings highlight the possibility of using selective α4β2* nAChR PAMs to enhance $GABA_AR$-mediated neurotransmission in human epilepsy.

P2.3

Targeted over-expression of multiple $GABA_A$ receptor subunits increases the sensitivity to GABAergic anti-seizure medications

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In spite of the many available anti-seizure medications (ASMs) about one-third of epileptic patients do not achieve a complete control of their seizures. Drug-resistant focal epilepsies represent an accessible target for gene therapy since the direct injection of the vector in the brain parenchyma can induce the expression of the therapeutic gene(s) in the brain area that generates focal seizures. Attempts in this direction have been made with genes that can modify cell function and control hyperexcitability, modulating some channels, neurotransmitters or receptors.
Here we propose to overexpress endogenous $GABA_A$ receptors by using a lentiviral vector in order to potentiate the response to clinically used ASMs. Therefore, we characterized GABA-evoked currents in *Xenopus* oocytes microtransplanted with hippocampal membranes obtained from mice injected with lentiviral vectors.
We found that the upregulation of benzodiazepine- and barbiturate-sensitive $GABA_A$ subunits induced an enhancement of potentiation of GABA-evoked currents after the treatment with diazepam and phenobarbital. These results suggest that upregulating $GABA_A$ receptors' subunits involved in the binding of ASMs may be a promising therapeutic opportunity for the treatment of drug-resistant epilepsy.

P2.4

Inhibitory transcranial magnetic stimulation reduces side effects of chronic Levodopa in experimental parkinsonism

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Dopaminergic medications are the most used drugs to treat the symptoms of Parkinson's disease (PD), and Levodopa is still widely considered the "gold standard" therapy. Despite beneficial effects, however, chronic Levodopa treatment is associated with motor fluctuations, wearing-off, and the development of dyskinesia. In experimental models, the dyskinetic condition is associated with impairment in corticostriatal bidirectional synaptic plasticity, alterations of NMDA receptor (NMDAR) subunit ratio, and post-synaptic density composition. In the last decades, inhibitory magnetic stimulation protocols such as continuous Theta Burst Stimulation (cTBS) have been suggested as a possible treatment for hyperkinetic movement disorders without providing a mechanism.
Here, we explored the therapeutic effects of two-week cTBS and Levodopa *in vivo* combined treatment in a symptomatic PD rodent model. Co-treatment prevented NMDAR-dependent plastic abnormalities in the dorsolateral striatum and reduced the incidence and intensity of dyskinetic behaviors. RNA-scope and flow cytometry analyses demonstrated that renormalization of glial reactivity and reduction of inflammatory markers occur in animals co-treated with cTBS showing no dyskinetic phenotype.
These findings suggest that cTBS protects corticostriatal synapses from the side effects of Levodopa therapy in a PD model, confirming the anti-inflammatory potential of noninvasive magnetic stimulation when appropriate patterns are chosen.

P2.5

Age dependent alterations in cortico striatal synaptic plasticity of transgenic mice expressing neuromelanin

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Among the neurodegenerative diseases Parkinson's Disease (PD) is one of the most common. It's been suggested that accumulation of neuromelanin (NM) in dopamine (DA)-releasing neurons of the substantia nigra may be the cause of their degeneration, thereby reducing DA release in target areas like the striatum.
We investigated possible alterations of synaptic plasticity in the striatum of Tg-TH-hTyr (hTyr) mice. These mice express the human tyrosinase enzyme inducing the production of NM in catecholaminergic neurons. Extracellular recordings were performed to measure synaptic plasticity of the cortico-striatal input. Induction of long-term depression (LTD) was defined as reduction of the synaptic response by at least 20% relative to control, 1h after the train.
In 6-7-month-old wild type (WT) mice LTD was induced in all recorded slices, while in 15-17-moths old WT mice the same protocol did not always induce LTD. Moreover, when LTD was induced, the degree of depression was higher in 6-7 than in 15-17 months old mice.
In hTyr we still observed a similar difference in the degree of depression between the two different ages, however, LTD was less pronounced that in WT at both ages.
In conclusion, cortico-striatal plasticity is subject to an age-dependent physiological reduction, however, NM accumulation leads to an acceleration of this process, exacerbating the loss of cortico-striatal synaptic plasticity at younger age, possibly for a reduction in the efficacy of the DAergic input.

P2.6

Neural processes underlying motor control and decision making when acting with others

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Our social behavior is mainly expressed by the ability to act together with other agents. Joint actions (JA) enable achieving goals unattainable when acting alone, but often requires a behavioral cost, that must be evaluated when choosing between acting individually or in coordination with others. Here, we study in a series of experiments the neural processes underlying these choices and motor control during joint behavior. To this aim, couples of macaques were trained to act alone and in pairs, or free to choose between individual (IA) and joint action (JA), to obtain certain amounts of rewards, associated to each action type. The results showed that macaques discriminate between offers, with a stark action bias in favour of IA. Choices were subdued to the payoff value, which took into account the cost of JA, besides the reward amount offered. Local Field Potentials recorded from macaque premotor cortex showed a clear representation of the goodness of motor coordination. Single-unit activity (n=366) recorded from the macaque prefrontal cortex (PFC), was mostly modulated during the choice phase, by future action-type and reward quantity, and suppressed during action planning and execution. Crucially, we found neurons activated only when monkeys choose to act together and cells representing the prediction of other's choice. Our results support the role of the prefrontal cortex in good-to-action transformation, when choosing to act in a social context.

P2.7

Functional and morphological alterations in mouse locus coeruleus noradrenergic neurons expressing neuromelanin granules

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The dark-brown pigment neuromelanin (NM) accumulates with age within human catecholaminergic brain nuclei mainly due to excessive cytosolic dopamine and noradrenaline not sequestered into synaptic vesicles that are oxidized into NM. By contrast, common laboratory animals such as rodents, lack NM. Neurons with the highest NM levels, including midbrain dopaminergic neurons from *substantia nigra pars compacta* (A9), ventral tegmental area (A10) and pontine noradrenergic neurons of the locus coeruleus (LC/A6), are those preferentially vulnerable in Parkinson's disease (PD), being LC one of the first nuclei to become affected. We took advantage of a recently developed mouse model over-expressing human NM-synthetizing enzyme tyrosinase (hTyr), which displays NM accumulation in all catecholaminergic brain nuclei. Electrophysiological recordings in ex-vivo LC slices form 6-7 month old mice indicate that NM-expressing neurons display higher membrane capacitance and resistance and increased Ca^{2+}-activated potassium conductance. In addition to these alterations, LC neurons from 15-18 months old mice display lower ability to fire action potentials in response to depolarizing stimuli. Histological analysis revealed massive loss of LC neurons in old hTyr mice despite surviving cells do not show soma size/perimeter alterations. Our data suggest that NM deposition is detrimental for LC neurons, causing progressive alteration of neuronal excitability and neurodegeneration.

P2.8

Morpho-functional alterations and miRNome dysregulation in the retina of a mouse model of Alzheimer's disease: paving the way for new strategies of gene therapy

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Alzheimer's disease (AD) is a neurodegenerative disorder for which effective diagnostic tools and treatments are still missing. Gene therapy has been shown to provide potential benefits in neurodegenerative disorders, but therapeutic efficacy depends on knowledge of the disease pathogenesis. Most evidence about mechanisms involved in AD progression derives from animal models such as 5xFAD. We performed a longitudinal analysis of retinal morpho-functional and molecular alterations in 5xFAD mice. Using electroretinogram and optical coherence tomography, we observed a progressive loss of visual function followed by retinal thinning starting at 6 months of age. Concurrently, we demonstrated retinal beta-amyloid and phospho-Tau accumulation correlating with photoreceptor and ganglion cell degeneration, and the progressive up-regulation of oxidative and inflammatory markers. This suggests that the retina might be considered a reliable tool to monitor AD progression and a benchmark for testing novel treatment efficacy. Therefore, we analyzed the whole retinal miRNome, highlighting the dysregulated expression of miRNAs in 5xFAD mice. These data might pave the way for a possible novel gene therapy strategy based on reinstating miRNA homeostasis, to be preliminary tested in the easily accessible retina and then, if successful, in the brain.

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P2.9

BTK inhibitors modulate remyelination in Multiple Sclerosis

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Myelin repairing mechanisms in people with MS are altered and demyelinated axons are exposed to stressors, leading to neurodegeneration especially in progressive MS. Nowadays, remyelination is a therapeutic unmet need in MS. Bruton's tyrosine kinase inhibitor (BTKi), such as Ibrutinib, are thought to be able to delay MS progression. To date, remyelination has been tested in vitro using prenatal models. Here, we test Ibrutinib effects on remyelination proposing a novel in vitro model using cells obtained from post-natal mice. Firstly, we verified that cultures by post-natal mice remyelinated after LPC-mediated demyelination. Then we assessed that Ibrutinib improved remyelination in mouse model of de- and re-myelination. It is known that Ibrutinib effects require a crosstalk between BTK-expressing cells and OPCs. We evaluated extracellular vesicles (EVs) release from microglial cells but no differences in quantity release was observed. We hypothesize that OPCs/microglia interaction lays on EVs content modification induced by Ibrutinib. This is the first study showing the BTKi positive effects on remyelination in an animal in vitro model allowing us to quantify remyelination modulated by treatments and to reduce animal sacrifices to pursue the 3R.

P2.10

Neural fingerprints of grasping actions in the monkey premotor cortex in head-fixed, head-free, and freely moving contexts

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The premotor cortex is a key region for the planning and control of grasping in primates, which typically occur in a variety of conditions and contexts. However, so far, the current knowledge on the neural underpinnings of grasping actions is essentially derived from neural recordings in head-fixed monkeys. Here, we recorded single unit and local field potentials (LFPs) from the primary (M1) and premotor (PM) cortex of two monkeys while they grasped food morsels in head-fixed, head-free, and freely moving contexts. Pre-screening of LFP modulation in different frequency bands using both LFP amplitude and power showed that the 1–10 Hz band exhibits consistent modulation patterns specific for different sets of channels. By exploring the reproducibility of the clustering involving 128 simultaneously recorded channels in all contexts, we found similar modulation patterns for reach-to-grasp in specific groups of channels during head-fixed and head-free contexts, but a poor match with the freely-behaving context. Likewise, single-unit activities exhibit reproducible patterns between head-fixed and head-free, but not freely moving, contexts.
Our findings reveal context-dependent neural fingerprints of grasping in functionally distinct subregions of the PM, suggesting that coordination between these subregions may underlie flexible, context-dependent construction of cortical motor synergies for grasping.

P2.11

Dual dose-dependent effects of palmitic acid (PA) on viability and maturation of Human Oligodendrocytes

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Palmitic acid (PA; 16:0) is the most common long-chain saturated fatty acid endogenously synthesized and found in the diet. PA plays physiological roles in the CNS. Specifically, oligodendrocytes (OLs) use it as a precursor in the myelin formation. However, cellular response of OLs to PA is not well understood. Our data show that an excess of PA administration leads to detrimental effects on the immortalized human OLs cell line, MO3.13, resulting in decreased cell viability. In contrast to other cell models, in MO3.13 PA is able to reduce intracellular and mitochondrial reactive oxygen species (ROS) levels in a dose-dependent manner and protect cells from the pro-inflammatory cytokine TNFα-dependent increase of ROS levels. We also found that PA activates the Nuclear Factor Erythroid 2-Like 2 (Nrf-2) antioxidant pathway and its downstream genes, suggesting that the decrease in ROS levels could be the consequence of an adaptive cellular response to PA load. Furthermore, our data show that PA influence the early stages of OLs maturation by reducing their migration and proliferation, while in the late stages of maturation, PA appears to have a dual role. Indeed, high doses of PA lead to an activation of the intrinsic apoptotic pathway, while low doses appear to promote differentiation.

P2.12

Effect of exercise training on postural control in people with diabetes

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Exercise is a key component of diabetes prevention and treatment. During self-motion we are exposed to optic flow patterns whose spatial and temporal properties directly influence the body sway. We aimed to study the effect of an exercise program on the activation of postural muscles and balance control in 12 diabetic people who followed a 12 months exercise program (age 59,1±10,2, BMI 29,3±5,7) and in 12 diabetic subjects with sedentary habits (age 59,4±12,1, BMI 32,5±5,8). During the view of optic flow stimuli, EMG signals were recorded bilaterally from the tibialis anterior (TA) and soleus (SOL) while the subjects stood on two force platforms to obtain the center of pressure (COP) displacement. Experiments were performed in the dark. Radial expanding optic flow stimuli were projected on a screen covering 135x107° of visual field. Recordings have been performed at month 0, 6 and 12 and analyzed by repeated measures ANOVA. EMG analysis showed a significant difference for optic flow stimuli (TA $p=0.036$; SOL $p=0.007$) and laterality (SOL $p=0.007$). Stabilometric data showed significant interaction effects for group x stimuli x time ($p<0.001$) and stimuli x sex x time ($p<0.001$) in the COP antero-posterior direction, and for laterality ($p=0.028$) in the COP medio-lateral direction. Results suggest that diabetic people who exercise regularly have a better body balance than sedentary counterparts. This augmented control improved across the time of exercise.

P2.13

Sex affects resting-state electroencephalographic rhythms in patients with dementia due to Parkinson's and Lewy body diseases

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Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB) are more prevalent in males than females. Furthermore, they typically showed abnormally high delta (< 4 Hz) and low alpha (8-10 Hz) rhythms from resting-state electroencephalographic (rsEEG) activity. Here, we hypothesized that those abnormalities may depend on the patient's sex.
An international database provided clinical-demographic-rsEEG datasets for cognitively unimpaired older (Healthy; N = 49; 24 females), PDD (N =39; 13 females), and DLB (N =38; 15 females) participants. Each group was stratified into matched female and male subgroups. The rsEEG rhythms were investigated across the individual rsEEG delta, theta, and alpha frequency bands based on the individual alpha frequency peak. The eLORETA freeware was used to estimate cortical rsEEG sources.
In the Healthy group, widespread rsEEG alpha source activities were greater in the females than in the males. In the PDD group, widespread rsEEG delta source activities were lower and widespread rsEEG alpha source activities were greater in the females than in the males. In the DLB group, central-parietal rsEEG delta source activities were lower, and posterior rsEEG alpha source activities were greater in the females than in the males.
These results suggest sex-dependent hormonal modulation of neuroprotective-compensatory neurophysiological mechanisms in PDD and DLB patients underlying the generation of rsEEG delta and alpha rhythms.

P2.14

Correlation between cortical plasticity and cognitive skills in aged subjects

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Cognitive skills are subtended by cortical plasticity. In the human primary motor cortex, plasticity is commonly studied through paired associative stimulation (PAS). Currently, an index based on responsivity to PAS and association between cortical plasticity and cognitive skills is lacking. This study investigates which PAS index better discriminates between responders (RR) and no-responders (NR) to PAS and its correlation with cognitive skills. Sixty-two healthy subjects (67.8±6.5 y.o., 29 males) were enrolled. Motor-evoked potentials (MEP) were recorded at baseline and after 0, 10, 20 and 30 minutes from PAS delivery. MEP amplitude ratios at each time point and at baseline were used to calculate the area under the curve (AUC), grand average (GrA) and curve concavity (CC), which were used as PAS indexes. The Montreal cognitive assessment (MoCA) was used to assess cognitive skills. MoCA significantly correlated with CC (p=0.013), but not with AUC (p=0.111) and GrA (p=0.091). Only when CC was used to discriminate PAS responsivity, MoCA was significantly higher (p=0.014) in RR (27.2±2.3) than NR (25.7±2.1). In conclusion, CC may represent a potential plasticity index to describe cognitive skills in humans, with a possible practical application in patients with cognitive impairment.

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P2.15

Default mode network and posterior resting-state electroencephalographic alpha rhythms in Alzheimer's disease patients

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Alzheimer's disease dementia (ADD) is the most common neurodegenerative disorder. Previous studies in ADD patients showed significant neurodegenerative processes as revealed by reduced gray matter volume in the cerebral cortex, including the default mode network (DMN) from structural magnetic resonance imaging (sMRI). Also, abnormalities in the resting-state eyes-closed electroencephalographic (rsEEG) alpha rhythms reflecting vigilance dysfunctions were observed. Here, we tested the hypothesis of a significant relationship between these neurodegenerative and neurophysiological readouts, thus suggesting the sensitivity of rsEEG alpha rhythms to intrinsic AD processes.
Clinical, sMRI, rs-functional MRI, and rsEEG rhythms in demographic- and age-matched ADD (N = 45), subjective memory complaint (SMC, N = 160), and healthy elderly (Nold, N = 40) persons were available from an international archive. Individual alpha frequency peak was used to determine the alpha frequency band. Freeware platforms served to estimate rsEEG sources and analyze MRI data.
The results showed (1) lower DMN volumes and posterior rsEEG alpha rhythms in the ADD than Nold participants and (2) a positive association of the functional connectivity of basal forebrain-DMN and the posterior rsEEG alpha rhythms in the SMC participants negative but not positive to amyloid PET biomarkers.
A prominent relationship was observed between DMN and the posterior rsEEG alpha rhythms across physiological and AD-related aging.

P2.16

Role of cerebellar network excitability and plasticity in the pathophysiology of dystonia

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DYT1 is an autosomal dominant form of dystonia, linked to a deletion in the TOR1A gene. The basal ganglia have been found to play a crucial role in the development of dystonia in the TOR1A$^{\Delta GAG}$ murine model. Experimental evidence has also indicated that the cerebellar cortex is likely to play a pivotal role in the pathophysiology of this disorder. We investigated cerebellar cortex activity and synaptic plasticity by performing extracellular recordings in acute parasagittal cerebellar slices with a high-density multielectrode array that allows to simultaneously record the activity of several neurons distributed in the whole network. We acquired spontaneous activity and stimulus-induced responses to mossy fiber stimulation, such as local field potentials in the granular layer. The network activity was also characterized after the induction of long-term synaptic plasticity at the mossy fibers-granule cell relay in both WT and TOR1A$^{\Delta GAG}$.
A separate set of experiments was performed using whole-cell patch-clamp recordings to investigate the passive membrane properties and intrinsic excitability of cerebellar granule cells and Purkinje cells in WT and TOR1A$^{\Delta GAG}$.
These data will be crucial to understand the role of different neuronal types in shaping the cerebellar cortex activity in the DYT1 mouse model compared to the WT, helping to uncover differences in signal integration and processing in this pathological model.

P2.17

Conjugated polymer nanoparticles rescue vision loss in a mice model of retinal degeneration

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Retinal dystrophies such as *Retinitis Pigmentosa* (*RP*) are among the major causes of inherited progressive blindness in developed countries, affecting 5.5 million patients, for which treatments are in demand. Retinal prostheses have been explored as a possible solution to restore visual functions. Recently we reported that conjugated polymer nanoparticles (CP-NPs) effectively preserve visual functions when injected subretinally in the aged Royal College of Surgeons rat model of *RP*. This model bears a mutation that impairs the retinal pigment epithelium (RPE) function with the subsequent photoreceptor degeneration. Here, we subretinally injected CP-NPs in the pigmented retinal degeneration 10 mouse model, characterized by a missense mutation that directly affects rod photoreceptors, to verify the effect of the CP-NPs when the RPE phagocytotic activity is preserved.
Our results further prove that CP-NPs reinstate visually driven activities at both subcortical and cortical levels even in a pigmented model of retinal degeneration, with a healthy and functional RPE, while no recovery is present in sham-injected animals. These results highlight the clinical potential of our NPs for restoring visual functions in fully degenerated retinas, an advanced stage of the disease mimicking the clinical conditions of patients affected by *RP* subjected to prosthetic interventions.

P2.18

The gut-brain axis: the role of the microbiota as a mediator of the enriched environment

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Commensal microbes in the gut play a crucial role in the host's health and disease. The gut-brain axis model seeks to elucidate the connections between these two systems and their mutual influence. The positive effect of an enriched environment in the brain is known, and in our recent study, we found that an enriched environment (EE) modified both the microbial profile and metabolite concentration in animals. Administration of short-chain fatty acids (SCFAs) resulted in behavioral and molecular changes in the brain similar to those observed under EE conditions. We then focused on the microbial aspect, using a specific bacterial cocktail (*Bacteroids gallinarum, Parasutterella excrementihominis, Caterbacter hongkongensis, Alistipes senegalensis, Clostridium kluyveri*) highlighted in EE homing to replicate the observed effects using metabolites. Additionally, we performed fecal material transplantation (FMT) from EE donors to determine if the modifications were due to the interaction between microbes and metabolites. Our results showed that both treatments altered the gut microbial community and improved anxiety-like behaviors, along with increased hippocampal neurogenesis and neurotrophin modulation.

P2.19

Manipulating the sympathetic mediated inhibition of immunity to prevent the development of health-care associated infections

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Background. Healthcare-associated infections (HAIs) significantly impact morbidity, mortality, and healthcare costs. This study examined if pharmacological prophylaxis targeting sympathetic reflex control of immunity could offer a novel approach to combat HAIs.
Methods. The study involved animal experiments and a retrospective analysis of orthopedic surgery patients in Romagna, Italy. Young female pigs were given intravenous *E. coli* and divided into two groups: propranolol-treated (non-selective β-blocker; 3mg/kg; 3x/day orally) and vehicle-treated, starting two days before infection until the experiment's end. Parameters assessed included bacteremia, serum cytokines, biochemical profile, complete blood count, lactate, glycemia, and flow cytometry. Additionally, a retrospective analysis of 92,649 orthopedic surgery patients examined the impact of non-selective β-blockers on HAI development using conditional logistic regression, matching infected and non-infected patients by gender, age, and comorbidities.
Results. Propranolol-treated pigs showed a stronger immune response, clearing bacteria faster than vehicle-treated pigs. The retrospective analysis indicated that patients on non-selective beta-blockers had a 71.7% lower risk of developing HAIs.
Conclusion. These results suggest that targeting sympathetic reflex control of immunity through pharmacological prophylaxis can reduce HAIs, including those caused by multi-drug resistant organisms, in hospitalized patients.

P2.20

Microglia across neurodegenerative diseases: role of EVs-miRNA in neuroinflammation

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Extracellular vesicles, EVs, a heterogeneous population of membrane vesicles, which contain, and transfer bioactive molecules play a role in many of the major pathological pathways altered in neurodegeneration, including Aβ aggregation, neuroinflammation, synaptic transmission, cell death, and senescence. Of note, most EV effects are mediated by encapsulated miRNAs.
On the one hand, with the progression of neuropathology, the inflammatory response of microglia can influence the expression of EV-miRNAs, whose release could promote neuroinflammatory processes.
On the other hand, EVs from neural stem cells, (NSC-EVs) have been explored for their ability to modulate neuroinflammation and neuronal-glial functions in neurodegenerative disorders.
In this regard, we proved that microglia have the capacity to self-sustain its active state, by releasing vesicular and non-vesicular pro-inflammatory factors contributing to the spreading of neuroinflammation.
We therefore demonstrated that the intracellular misregulation of selective inflamma-miRNAs in response to inflammatory stimuli is mirrored in the composition of EVs-miRNAs, proving their role in exacerbating the neuroinflammatory response *in vitro*.
Additionally, we aim to identify potential immunomodulatory NSC-EVs-miRNAs to prove that the contribution of EVs-miRNAs in neurodegenerative pathologies reflects the characteristic of the cells from which they are released.

P2.21

Natural Killer cells modulate peri-tumoral neuron activity in Glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor, representing a clinical challenge due to its infiltrative nature and resistance to therapy. GBM cells release several cytokines that silence the cytotoxic activity of infiltrated immune cells, creating an immunosuppressive/pro-tumoral environment. In this scenario, Natural Killer cells invasion is weak, and tumor cells attenuate NK-mediated killing. Besides, it was reported that peri-tumoral neuronal activity fosters malignant behavior of GBM, shaping excitation/inhibition balance and building chemical synapses between presynaptic neurons and postsynaptic tumor cells, supporting tumor invasion and growth. In this scenario, we investigated NK cells' impact on neuronal activity in murine GBM models. At first, we described that both *in vitro* then in GBM-bearing mice, NK cells contact peri-tumoral neurons at synaptic and soma levels. Moreover, qRT-PCR analysis unravelled that peri-tumoral neurons increase the expression of chemokines (cxcl10 and cxcl9) able to recruit immune cells, and express membrane proteins that trigger the cytotoxic activity of infiltrated NK cells, inducing neuronal death. We further described a neuromodulatory role of NK cells in peri-tumoral area, since whole-cell patch clamp recordings revealed heightened firing frequency of excitatory peri-tumoral neurons in NK cell-depleted GBM-bearing mice. This study sheds light on GBM pathophysiology, offering insights into potential therapeutic targets.

P2.22

A key molecular regulator of neuronal excitability and network stability: the interaction between PRRT2 and Nav1.2

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Proline-rich transmembrane protein 2 (*PRRT2*) is the single causative gene for pleiotropic paroxysmal disorders. It encodes for a neuron-specific protein with multiple functions, including the modulation of voltage-activated Na^+ (Nav) channels and synaptic properties (Valente et al., 2016, 2018). PRRT2 loss-of-function mutations correlate with paroxysmal disorders, and lack of PRRT2 function has been associated with increased excitability, leading to network instability. Indeed, PRRT2 interacts with specific subtypes of Nav expressed by excitatory neurons. PRRT2 depletion was found to increase Na^+ currents and, conversely, its presence reduces the Nav activity modulating the biophysical properties of Nav1.2/1.6 subtypes (Fruscione et al., 2018). Using specific missense mutations in the channel protein, we try to uncover the molecular interactors of PRRT2 and Nav1.2. Using computational tools, several amino acids clusters in the Nav1.2 alpha subunit were identified, which could potentially affect the interaction with PRRT2. Channels mutated at these residues are heterologously expressed and functionally studied by patch-clamp experiments. Results will provide an additional useful framework for investigating the interaction between PRRT2 and Nav1.2. (Italian Ministry of University and Research (funded by MIUR, PRIN 2022MPCKWW to PV).

P2.23

Characterization of mitochondrial dysfunction and therapeutic potential of Nrf2 modulation in early-stage Parkinson's Disease using Pink1$^{-/-}$mice

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Mitochondrial dysfunction is increasingly recognized as a critical factor in Parkinson's disease (PD) development. The nuclear erythroid 2-related factor 2 (Nrf2) regulates mitochondrial function and cellular biogenesis, resisting oxidative stress. Consequently, Nrf2 represents a potential novel target for disease-modifying therapies for PD. Since 2007, PTEN-induced kinase one knockout (PINK1-/-) mice have been widely used as an experimental model for early genetic parkinsonism linked to recessive PINK1 gene mutations. While extensively characterized at ages 2, 3, 6, and 9 months, showing deficits in bidirectional corticostriatal synaptic plasticity, spontaneous locomotor activity, and vocalizations, little is known about alterations at earlier ages, such as postnatal day 30 (P30). This project aims to conduct a detailed behavioral and synaptic characterization of PINK1-/- mice at 30-34 days old. Using the Seahorse XF instrument, we evaluated the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) levels. Additionally, after four weeks of treatment, we investigated the effect of Lisosan G, a dietary supplement that directly modulates Nrf2, on the altered behavioral, biological, and physiological parameters in PINK1-/- mice. The study aims to identify pathways involved in mitochondrial energy deficits and evaluate Lisosan G's efficacy as an additive strategy to existing PD treatments.

P2.24

Virtual brain simulations unveil hidden physiological parameters regulating network dynamics in dementia

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Assessing synaptic and neuronal parameters in the human brain still remains impractical. Here we show that a recent technology, virtual brain modelling, can shed light on the microscopic underpinnings of networks activity. The virtual brain is made of nodes (brain areas) and edges (fibers tracts) obtained from MRI data. In this brain "avatar" nodal activity can be represented using different models, i.e. the Wong-Wang neural mass, which allow to estimate the NMDA and GABA receptor-mediated activity and obtain subject-specific information about excitation/inhibition (E/I).
In this work we used virtual brain modelling to investigate resting-state network dynamics in healthy subjects and in Mild Cognitive Impairment (MCI) at different progression levels. Our results show that glutamatergic recurrent-excitation shapes the attention network functional topology, especially in MCI patients negative to Aβ and τ biomarkers. On the other hand, cognitive worsening determines structural and functional reorganization of the limbic network, accompanied by nodes hypersynchrony. The high correlation (R^2 index > 70%) found between the combined topological and E/I description and subject-specific neuropsychological scores validate the clinical utility of this characterization, opening new perspectives for a deeper understanding of brain mechanisms underlying cognitive impairment progression.

P2.25

TMEM151A, a new causative gene in Paroxysmal Kinesigenic Dyskinesia

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TMEM151A, an almost unknown gene, has been recently associated to Paroxysmal Kinesigenic Dyskinesia (PKD), an autosomal dominant movement disorder which previously was mostly associated to PRRT2 gene mutations. Interestingly, the drug treatment of choice for PKD caused by TMEM151A or PRRT2 mutations is Carbamazepine, a sodium channel blocker. Indeed, PRRT2 has been shown to control neuronal excitability by modulating voltage gated sodium channels. Both PRRT2 and TMEM151A are membrane associated proteins expressed in the nervous system. To gain insight on TMEM151A function we investigated the effects of some pathological mutations of TMEM151A by in vitro assays. We show that some of them cause alteration in the protein expression, suggesting a possible loss of function mechanism, similarly to what frequently happens with PRRT2 pathological variants. Therefore, our hypothesis is that PRRT2 and TMEM151A could share similar pathophysiological mechanisms. To address this point, we are studying TMEM151A topology and expression as well as its role in neuronal development and excitability. To examine TMEM151A membrane topology, we modelled the protein structure through in-silico 3D simulations that have been validated by live-labelling immunofluorescence and electron microscopy assays. This may help to understand the physio-pathological mechanisms at the basis of these paroxysmal disorders and to get a step forward for the identification of new targeted therapeutic strategies.

P2.26

Natural Killer cells prime the synaptic pruning by microglia necessary for normal memory formation

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Natural Killer (NK) cells play a critical role in priming microglia, essential for memory formation. RNA sequencing showed that microglia from mice treated with a NK-depleting antibody had increased expression of inflammatory genes and altered synaptic modulation genes, particularly in the hippocampus. NK depletion caused microglia to become more ramified, with increased pre- and post-synaptic contacts and decreased expression of synaptic pruning genes. Consequently, NK depletion impaired spatial memory performance in behavioral tests. Hippocampal analysis revealed that NK-depleted mice had higher dendritic spine density, fewer mature spines, and fewer microglia-neuron contacts compared to controls. We hypothesized that interferon-gamma (IFN-γ) mediates these effects and conducted behavioral experiments on mice treated with an IFN-γ blocking antibody. This treatment replicated the effects of NK depletion on behavior, spine density, maturation, and microglia-neuron contacts. To validate our hypothesis, we treated mice with a dendrimer containing siRNA targeting the IFN-γ receptor 1 in microglia, which also replicated the effects observed in NK-depleted mice. Since STAT1 is a downstream messenger in the IFN-γ pathway, we conducted tests with STAT1 knockout mice that showed similar effects to NK depletion and IFN-γ blockade. In conclusion, NK cells, through IFN-γ release, are crucial for proper synaptic pruning by microglia, necessary for memory formation.

P2.27

Brain structural and functional connectivity biomarkers for early detection of alzheimer's disease

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Cortical connectivity networks deteriorate early in Alzheimer's disease (AD). Noteworthy, recent studies highlighted the impact of neuroinflammation and its interplay with extracellular matrix (EM) proteins, such as metalloproteases (MMPs) on neuronal activity, suggesting new diagnostic methods and potential therapeutic strategies.
Here we aim to characterize changes in brain connectivity in a sporadic AD mouse model, correlating these changes with signs of neurodegeneration. We used a mouse model of AD obtained by recurrent Herpes Simplex virus-1 infections. AD mice and their respective controls underwent cognitive tests and were recorded using local field potentials (LFP) to create a behavioral and brain connectivity correlation. Results showed that AD mice in the early phase of the pathology, exhibited only recognition memory deficits that worsened in the more advanced phase, when spatial memory impairment and motor deficits were also seen. In the late phase, AD mice compared to controls, showed signs of neuroinflammation, as the levels of the anti-inflammatory cytokine, IL-10 decreased and pro-inflammatory TNF-α increased in different brain areas, along with changes in the NF-kβ/GSK3β pathway and altered expression of MMP2, MMP9 and collagens. Notably, preliminary LFP analysis showed differences in cortical connectivity between AD and control mice, suggesting a link between altered connectivity, functional impairment, EM remodelling and neuroinflammation.

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P2.28

Lithium-charged gold nanoparticles: a new tool for the *in vitro* and *in vivo* modulation of Glycogen Synthase Kinase 3

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Glycogen Synthase Kinase 3 (GSK-3) is a hub kinase whose activity regulates hundreds of cellular processes, both physiological and pathological. Indeed, mood disorders and some neurodegenerative diseases are linked to GSK-3 activity deregulation, whereas entry and replication of many neurotrophic viruses depend on host cells' GSK-3 activity. Lithium (Li^+) is a potent GSK-3 inhibitor, but it faces challenges in systemic use due to its high toxicity, especially for kidneys and thyroid. Nowadays, Li^+ salts are used for neuropsychiatric disorders treatment, but safe doses are ineffective against other GSK-3-dependent illnesses, which require higher toxic Li^+ doses.
We developed an efficacious tool for Li^+-based modulation of GSK-3 activity which consists of gold nanoparticles (AuNPs) functionalized with glutathione and charged with Li^+ in the outer corona (LiG-AuNPs). Once aggregated, LiG-AuNPs can internalize cells thus determining an efficient intracellular release of Li^+ and a significant GSK-3 inhibition, even at Li^+ concentration lower than normally used in classical salt formulations. If intranasally administered in a mouse model, LiG-AuNPs reach the brain and modulate GSK-3 activity *in situ*, bypassing the systemic administration and without significantly affecting plasma Li^+ levels. Collectively, LiG-AuNPs offer a promising method for brain-specific Li^+ delivery, potentially beneficial for treating GSK-3-dependent neurological diseases while avoiding the Li^+ side effects.

P2.29

Exploring basal locomotor activity and light-off visual-motor response in zebrafish larvae: influence of age, time of the day, and biological replicates

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The advent of commercial platforms using video tracking technologies has led to a significant increase in the study of zebrafish larval behaviour to assess neurotoxicity. Among the most used assays are basal locomotor activity (BLA) and visual motor responses (VMRs), but the influence of intrinsic and extrinsic factors remains unexplored. The present preliminary study aimed to validate the use of zebrafish larval behaviour as a reliable endpoint of neurotoxicity by investigating the influence of various variables that may affect the experimental results, such as the influence of age-dependent nervous system development (using larvae from 5 to 8 days post-fertilisation), time of day (8:00 am, 10:00 am, 12:00 noon, 2:00 pm, 4:00 pm, 6:00 pm and 8:00 pm) and biological replicates (three experiments conducted on different days) on BLA and VMR outcomes, for a total of 4004 analyses per behaviour across 143 larvae. A generalised least squares (GLS) random effects linear regression model was used to account for the influence of these variables, including their two-way and three-way interactions. The results of this study indicate that all three factors, along with their interactions, significantly influence both behaviours. These findings highlight the need for each laboratory involved in zebrafish behaviour analysis to test the influence of these factors under their specific experimental conditions to ensure the reliability and consistency of zebrafish larval behaviour data.

P2.30

Chronic exposure to low doses of benzene affects cognitive functions through the alteration of molecular mechanisms underlying brain plasticity

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Volatile organic compounds (VOCs) represent one of the main sources of air pollution. They include several classes of chemical compounds characterized by a higher tendency to evaporate even at room temperature, that can occur from both natural and anthropogenic processes. Among VOCs, benzene, which is widespread in the outdoor environment, is one of the most harmful since it can cause damage to multiple organs but its impact on brain function remains elusive. Here we intranasally administrated low doses of benzene (0.05 µg/die) to three-month-old C57Bl/6 mice for about 6 weeks. Then we performed some behavioral tasks, such as Novel Object Recognition (NOR) and Object Place Recognition (OPR) tests to evaluate the hippocampus-dependent cognitive function. We found that benzene administration impaired memory by inhibiting both the phosphorylation of CREB and the expression of BDNF in the hippocampi of treated mice. Moreover, immunofluorescence analysis revealed a significant increase of the immunoreactivity for GFAP, IBA1 and TMEM119, in the hippocampal tissues of treated mice. Collectively, our data reveal that exposure to low dose of benzene may impair memory by triggering neuroinflammation and affecting neuroplasticity in the hippocampus.

P2.31

Cortical neural synchronization mechanisms are abnormal in patients with dementia due to Parkinson's and symptomatic Huntington's diseases

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Background. Parkinson’s disease (PD) and Huntington’s disease (HD) are neurodegenerative conditions affecting the basal ganglia, leading to movement-related symptoms and potential cognitive decline or dementia. Resting-state electroencephalographic (rsEEG) rhythms reflect neurophysiological mechanisms associated with brain arousal fluctuations. The hypothesis proposes that rsEEG sources may show more pronounced abnormalities in patients with symptomatic Huntington's disease (S-HD) compared to those with dementia due to Parkinson’s disease (PDD).
Methods. Clinical and rsEEG data from 18 S-HD, 16 PDD, and 25 Healthy participants were collected and matched for demographics, education, and gender. eLORETA software estimated cortical rsEEG sources across various frequency bands.
Results. Results showed a decrease in the amplitude of posterior alpha (about 8-12 Hz) activities and an increase in widespread low-frequency bands (specifically delta, <4Hz, and theta, about 4-7 Hz) in both PDD and S-HD groups compared to the healthy group. Furthermore, in comparison to the PDD group, the S-HD group exhibited more pronounced reductions in rsEEG alpha 2 rhythms in the frontal and temporal regions.
Conclusion. These results suggest that rsEEG rhythms' cortical sources may reflect different abnormalities in core neurophysiological mechanisms underlying brain arousal in PDD and S-HD patients. These rsEEG markers could be clinically useful for disease staging, monitoring, and drug discovery.

P2.32

Muscle cell responses to a pan agonist of estrogen receptor-related receptors: an *in vitro* study of exercise physiology

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Regular exercise induces biochemical and structural adaptations in skeletal muscle, contributing significantly to health and longevity. Orphan nuclear estrogen receptor-related receptors (ERRs), characterized by ligand-independent exercise-induced constitutive transcriptional activity, play an important role in the exercise capacity of skeletal muscle. Interestingly, compound SLU-PP-332 has been identified as a pan agonist of ERRs capable of increasing mitochondrial function and muscle cellular respiration, representing an excellent opportunity to elucidate the mechanisms underlying muscle responses to exercise. Our study aimed to investigate the role of ERRs through an administration of SLU-PP-332 in myoblasts isolated from sedentary subjects in order to understand physiological adaptations to exercise. Primary cell cultures of human myoblasts isolated from muscle biopsies taken from sedentary patients undergoing hip arthroplasty for coxarthrosis were set up. Following administration of SLU-PP-332, quantitative and qualitative analyses were performed to assess reactive oxygen species (ROS) and protein expression. Treatment with SLU-PP-332 significantly reduced ROS production and modulated the expression of mediators involved in muscle metabolism, highlighting a crucial role of ERRs as mediators of muscle responses to exercise. This evidence could suggest ERRs as potential biomarkers whose targeting could aid in the management of a wide range of disuse-associated diseases.

P2.33

Translational potential of a new synthetic Sirtuin1-activating compound to rescue muscle performance and phenotypes in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a rare genetic disease caused by mutations in the dmd gene, inducing the loss of dystrophin protein, leading to muscle membrane fragility, degenerative inflammation, fibrosis, and regenerative impairment. Current research aims to develop new therapies to extend the lifespan and improve the quality of life for DMD patients, avoiding the side effects of the current approach with corticosteroids. Emerging findings suggest that mitochondrial dysfunctions are early defects in DMD muscle, leading to poor myofiber repair and contributing to muscle degeneration and necrosis. Therefore, maintaining a healthy balance in mitochondria can slow down skeletal muscle deterioration and enhance muscle function. Sirtuin 1 (SIRT1), a NADH-dependent class III histone deacetylase, is a stress sensor and metabolic regulator in mammals that affects cellular energy balance. It boosts mitochondrial biogenesis and fatty acid oxidation in skeletal muscle, ameliorating muscular pathology and exhibiting anti-inflammatory activity. Here, we assess the therapeutic potential of a new synthetic SIRT1-activating compound, SRT2104, in fly and mouse models of DMD, focusing on its effects on myofiber metabolism, regeneration, and muscle function. Results show that SRT2104 rescues muscle performance, structure, and phenotypes of DMD models, activates muscle metabolism, and restores mitochondrial respiratory capacity, confirming it is an attractive candidate for DMD treatment.

P2.34

Insulin-mimetic role of S-Allyl cysteine in C2C12 myotubes

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S-Allyl cysteine (SAC) is a natural compound of which black garlic is highly enriched, that received great attention as a 'multitasking tool' against several pathological conditions. Aim of this study is to evaluate the insulin-mimetic potential of SAC on C2C12 skeletal myotubes, from the viewpoint of its potential application to prevent insulin resistance.
To evaluate SAC metabolic effects, glucose uptake assays were performed with fluorescent microscopy (2-NBDG probe), using insulin as a positive control and S961 as insulin receptor inhibitor. Furthermore, SAC antioxidant activity was investigated with the ROS fluorescent probe CellROX™ Green, and the potential binding of SAC with the insulin receptor (IR) was predicted by molecular docking.
Our results show that SAC, like insulin, increases glucose uptake and this effect is missing in S961 pretreated cells, thus suggesting a direct interaction of SAC with the IR; these data are strengthened by molecular docking results. Moreover, as for the protective role against oxidative stress, SAC shows antioxidant activity in Menadione-stressed cells. Finally, preliminary data on insulin-resistant myotubes suggest that SAC could reverse this metabolic dysfunction.
In conclusion, SAC improves glucose uptake in skeletal myotubes, likely by a direct activation of the insulin receptor, and exerts antioxidant effects. Further experiments must be carried out to confirm the potential protective role of SAC in a condition of insulin resistance.

P2.35

Identification of sarcopenic obesity in adults undergoing orthopaedic surgery: Relationship between “a body shape index” (ABSI) and fat-free mass

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The increasing prevalence of obesity, especially visceral obesity, combined with a loss of muscle mass and strength can lead to sarcopenic obesity. This condition therefore requires increased attention in patients undergoing orthopaedic surgery, as the accelerated loss of fat-free mass (FFM) is associated with pain, mobility limitations and inflammation. In addition, loss of muscle mass is associated with prolonged hospitalisation, infection, non-infectious complications and disability.
Our study investigated the suitability of a waist circumference-based anthropometric measure, the body shape index (ABSI), for predicting sarcopenic obesity and the predictive power of the ABSI for the fat-free mass index (FFMI), a surrogate marker for lean body mass. A cross-sectional study in overweight and obese orthopaedic patients undergoing knee or hip and spine surgery was conducted in 120 women (aged 66.5 ± 9.6 years) and 89 men (aged 65.5 ± 7.8 years) with overweight and obesity at the Orthopaedic Hospital Valdoltra, Slovenia. The general anthropometric parameters body mass index (BMI) and ABSI = (WC/($BMI^{2/3}$x $height^{½}$) as well as body composition data were determined using bioelectrical impedance analysis (BIA).
We concluded, that sarcopenic obesity was most common in obese women scheduled for knee surgery. In addition, the ABSI independently predicted FFMI in women and represents a significant predictor of sarcopenic obesity.

P2.36

Changes in the spatial distribution of lumbar erector spinae activity are associated with force steadiness variation during sustained task

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Evidence on the neuromuscular factors affecting force control of trunk muscles is currently limited. This study aimed to explore the association between changes in the spatial distribution of lumbar erector spinae (ES) activity and force steadiness during a fatiguing task. Twelve young subjects (age: 26.8±3.2) performed isometric trunk extension at 30% of their maximal voluntary isometric force until task failure. High-density surface electromyography (HDsEMG) signals were recorded from the lumbar ES. The spatial distribution of lumbar ES activity was quantified using the x- and y-coordinates of the centre of gravity (CoG) of the HDsEMG amplitude map for the medial-lateral and cranial-caudal direction, respectively. Force steadiness was assessed using the coefficient of variation of force (CVf). Differences in the CoG coordinates and CVf were assessed between the start and the end of the fatiguing task. The association between the shift of the CoG along the x-axis and the change in CVf was examined using Pearson correlation. CVf increased at the end of the fatiguing task ($p<0.01$), indicating a greater force variability with muscle fatigue. A significant displacement of the CoG was shown in the medial direction (shift: -4.17±3.43 mm; $p<0.01$), which was positively correlated with the change in CVf ($R=0.57$; $p<0.05$). These results suggest that a progressive medial shift of lumbar ES activity is associated with improved force steadiness during sustained isometric trunk extension.

P2.37

The microtubules plus-end tracking proteins CLIP-170 mediates nuclear shape in Emery-Dreifuss muscular dystrophy

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Mutations in lamin A/C gene (*LMNA*) cause Emery-Dreifuss muscular dystrophy (EDMD). We aim to unravel the molecular and cellular mechanisms underlying EDMD in $Lmna^{p.H222P/H222P}$ mice, a model of this disease. We showed that a reduced acetylation of microtubules was responsible for altered microtubule organization in EDMD. Given the established role of microtubules in regulating nuclear shape, this study aims to elucidate the mechanisms by which abnormal microtubule organization contribute to nuclear elongation, a cellular phenotype of EDMD. CLIP-170, a microtubules plus-end protein, localizes at the poles of elongated nuclei in muscles fibres from $Lmna^{p.H222P/H222P}$ mice, while it localizes around the nuclei in the wild-type animals. CLIP-170's activities are contingent upon conformational changes. A folded conformation of CLIP-170 (phosphorylated form) dissociates from microtubule plus ends. Conversely, CLIP-170 in the open extended conformation (unphosphorylated form) binds microtubules with greater affinity. We investigated the effect of a neurosteroid that activates CLIP-170 by altering its conformation. We showed that this drug restores the nuclear shape in striated muscles of EDMD mice by removing CLIP-170 from the poles of elongated nuclei. These findings suggest that CLIP-170 plays a critical role in modulating nuclear shape in EDMD.

P2.38

The dependence on the afterload of the degree of thick filament activation in the heart

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According to the Starling Law of the heart the dynamic equilibrium in the circulatory system is maintained by a mechanism adapting the output of the ventricle in systole to its diastolic filling. At the level of the sarcomere, this mechanism is attributed to the length-dependent activation relating the systolic force to the end-diastolic sarcomere length (SL). Here, the mechanism is tested with sarcomere-level mechanics and X-ray diffraction to measure the degree of thick filament activation, the power and the SL in afterload contractions of intact trabeculae and papillary muscles of the rat ventricle. We find that, starting from the same end-diastolic SL, the number of myosin motors recruited from the OFF, ATP hydrolysis-unavailable state characteristic of the diastole, increases with the afterload (T), and almost saturates for $T \geq ½T_{max}$ (≈110 kPa, the maximum force attained in an isometric twitch, Reconditi *et al*. 2017, PNAS 114:3240-5). Accordingly, for $T < ½T_{max}$, the power developed during the afterload contraction is lower than that of the isotonic-release contraction and increases with T. At the organ level these results suggest that the aortic pressure, not the diastolic filling, determines the systolic performance, and that the ability of the heart to maintain the dynamic equilibrium in the circulatory system is based on downstream mechanisms which tune the systole energetics to the aortic pressure, independent of diastolic filling. Supported by MUR (Italy) and ESRF.

P2.39

Unveiling the biophysical mechanism of cardiomyocyte excitation-contraction coupling modulation by a membrane-targeted photoswitch

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The use of light to control the activity of living cells is a promising approach in cardiac research due to its unparalleled spatio-temporal selectivity and minimal invasiveness. Ziapin2, a newly synthesized azobenzene compound, has recently been reported as an efficient tool for light-driven modulation of excitation-contraction coupling (ECC) in hiPS-derived cardiomyocytes. However, the exact biophysical mechanism of this process remains incompletely understood.
To address this, we performed electrophysiological measurements in a more mature cardiac model, specifically adult mouse ventricular cardiomyocytes (V-CMs) and coupled our experimental data with an enhanced numerical model of murine action potential (AP) that accurately reproduces the alterations in cell capacitance and membrane potential induced by Ziapin2 photoisomerization.
Our *in vitro* results demonstrate that Ziapin2 can photomodulate cardiac ECC in mature V-CMs. We established a connection between Ziapin2-induced membrane thickness modulation and light-generated APs by showcasing the pivotal role of stretch-activated ion channels (SACs). Notably, our experimental findings, through pharmacological blockade, coupled with *in silico* observations, suggest that SACs with selective Ca^{2+} permeability might serve as the sole biological culprit responsible for the effect.
Taken together, these findings elucidate Ziapin2-mediated photostimulation mechanism and open new perspectives for its application in cardiac research.

P2.40

Selective inhibition of two aquaporin membrane channels, AQP3 or AQP9, impairs human PBMCs and neutrophil cell migration

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Peripheral blood leukocytes are able to move to the inflamed tissue, engulf and kill the invading bacteria. Rapid modification of cell shape and volume needs fast movements of water into or out of the cell, a function for which the aquaporins channels (AQPs) are thought to play a role. Recently, we showed that Aqp9 gene deletion normalizes oxidative stress and improves survival in murine models of sepsis. Here, we assess the expression and localization of AQP3 and AQP9 in human neutrophils and peripheral blood mononuclear cells (PBMCs) and evaluate their possible relevance in the phagocytosis and killing of *Klebsiella pneumoniae*, an opportunistic Gram-negative pathogen causing serious infections, including sepsis, and in cell migration. By immunofluorescence, AQP3 was localized at the plasma membrane of monocytes and at a lesser extent at that of lymphocytes while AQP9 was expressed in both PBMCs and neutrophils. A significant reduction of *K. pneumoniae* phagocytosis by monocytes after treatment with DFP00173 and/or RG100204, two potent and selective inhibitors of AQP3 and AQP9, respectively. By a transwell assay, RG100204 significantly impaired the cell migration of PBMCs with or without LPS stimulation while DFP00173 reduced the LPS-induced locomotion of PBMCs only after LPS exposure. Strong impairment of neutrophil migration was seen with the RG100204 both in presence or absence of LPS. Important roles for AQP3 and AQP9 are suggested in human leukocytes motility.

P2.41

Intercellular communication regulates mitochondrial Cx43 trafficking in astrocytes and glioma cell lines

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Connexin 43 (Cx43) is one of the most abundant connexins in central nervous system (CNS) playing crucial roles in gap junction (GJ)-dependent intercellular communication, and a number of channel-independent biological functions, including cell adhesion, regulation of gene expression, cancer pathogenesis and mitochondrial function. Cx43 has been localized in the mitochondria of different cell types, suggesting that this protein is also involved in energy homeostasis. However, its physiological role within these organelles remains largely unknown. To investigate the expression and role of mitochondrial Cx43 in astrocytes and glioma cells, we conducted a western blot analysis on immortalized human cell lines cultured at different density conditions and treated with lactate to induce a response based on different nutrients availability. We observed that mitochondrial Cx43 expression levels were significantly changed, depending on cell density and on the specific tested cell line. Our results indicate that cell density acts as a modulator of mitochondrial Cx43, suggesting that intercellular communication also affects Cx43 dynamic. These findings underline the role of Cx43 in mitochondrial function, confirming the importance of this protein as a sensor of cellular energy state and its influence on cellular metabolism.

P2.42

Effect of the exposure to a polyethylene terephthalate nanoplastics model (PET) on murine NHI-3T3 fibroblast cells

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Nanoplastics (NPs) have become a pervasive environmental pollutant widely present in all environmental matrices, raising concerns about health risks associated with ingestion, inhalation, and skin penetration. The skin represents one of the routes of exposure to NPs. This work aimed to study the effects of polyethylene terephthalate (PET) on NHI-3T3 cells, an immortalized fibroblast cell line obtained from mouse embryonic tissue, widely used as fibroblast model. Fibroblasts are essential for maintaining skin integrity, repairing wounds, regulating inflammation, and responding to environmental cues, making them key players in skin health and function.
Environmentally relevant model PET NPs were used, characterized by an intrinsic autofluorescence, and produced by a fast top-down approach based on mechanical fragmentation, a process close to the mechanical abrasion of microplastics occurring in the environment.
Results demonstrated PET NPs internalization in NHI-3T3 cells after 24h exposure, observed by live cell fluorescence microscopy. In parallel dose-dependent cytotoxicity and induction of intracellular oxidative stress were detected in exposed cells by MTT test and the ROS sensitive fluorescent probe CM-H_2DCFDA respectively.
In conclusion, this study provides information on the toxicity of PET NPs on NHI-3T3 fibroblast cells contributing to the knowledge of the risk associated with NP skin exposure.

P2.43

SARS-CoV-2 ORF3a protein is a water permeable channel that induces lysosomes swelling

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The SARS-CoV-2 ORF3a accessory protein plays an essential role in the virus release from the host cell during the infectious cycle. ORF3a mainly targets lysosomes where it promotes their inactivation through a deacidification process, an essential step for lysosomal exocytosis. However, the exact function of ORF3a is still elusive and debated. Seminal studies suggested ORF3a functioning as a viroporin (i.e., viral ion channel). However, a recent work disproved this conclusion. To unveil ORF3a function, we employed a multidisciplinary approach ranging from molecular dynamics (MD) to electrophysiology. The combination of ORF3a structural analysis through MD and patch-clamp recordings in HEK293 cells expressing ORF3a, unequivocally demonstrated that this protein is not a viroporin. Conversely, both MD simulation and video-imaging experiments designed to assess cell volume changes indicated that ORF3a may function as a water transporter. We also identified a putative selectivity filter for the passage of water formed by at least two asparagines. Mutation of asparagine 82 to either leucin (N82L) or tryptophan (N82W), abolished water permeation. Finally, ORF3a expression in HEK293 cells determined lysosomes swelling, an effect reverted by the N82W mutation. In conclusion, we identified a new possible function of the ORF3a protein as a water permeable channel that induces lysosomes swelling, an essential step inducing their deacidification and consequent inactivation.

P2.44

Mechanistic insights into estradiol-induced arrhythmias in long QT syndrome type 2

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Recent evidence indicates that the exogenous estradiol (E2) can act as a trigger of malignant cardiac arrhythmias in LQT2 patients. The aim of this study is to evaluate whether acute exposure to E2 causes cellular effects compatible with the facilitation of arrhythmias in the syndrome and to investigate the mechanisms. hiPSC-cardiomyocytes derived from a LQT2 patient (MUT) with arrhythmias induced by estrogen-based therapy and from a healthy donor (WT) were studied in control conditions or after 10 minutes pre-incubation with E2 (10 nM) with the patch-clamp technique. In MUT cells, rate-corrected action potential duration (c_APD) values were significantly increased compared to WT. E2 significantly shortened all c_APD values in MUT cells only. Despite APD shortening, E2 tended to increase the incidence of EADs. As all the observed E2 effects might result from changes in I_{CaL}, the latter was also evaluated. Treatment with E2 shifted the I_{CaL} activation curve towards positive potentials, while leaving the inactivation curve unchanged, thus narrowing the "I_{CaL} window". E2 accelerated the recovery from inactivation (RFI) of I_{CaL}, while the remaining I_{CaL} properties, including CDI, were unaffected. In conclusion, shortening of c_APD by E2 may result from the reduction of "I_{CaL} window" which is crucial for the AP plateau phase. The faster I_{CaL} RFI during the AP plateau might account for EADs facilitation despite APD shortening.

P2.45

Hypoxia-dependent upregulation of VEGF relies on β-adrenoceptor 3 signaling in endothelial and Müller cells: implications for ROP disease

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Retinopathy of prematurity (ROP) is characterized by a first phase of hyperoxia and a second one of hypoxia. Hypoxia stabilizes the α subunit of the hypoxia-inducible factor 1 (HIF-1α), resulting in increased activity of HIF-1. This latter promotes neovascularization stimulating the expression of vascular endothelial growth factor (VEGF), which in turn stimulates endothelial cell proliferation. In the retina, VEGF is mainly produced by Müller cells. The involvement of β-adrenoceptors (BARs) in VEGF production and retinal neovascularization has risen interest in the last decade. Here, using human Müller and retinal endothelial cells (MIO-M1 and hREC) we explored the functional role of BAR3 in hypoxia-dependent production of VEGF. In both cell lines, BAR3 levels are strongly upregulated by 24 hours of hypoxia, together with VEGF and HIF-1α. Conversely, the effects of hypoxia on BAR1 and BAR2 are negligible or nil. Interestingly, the hypoxia-dependent VEGF increase was prevented by BAR3 antagonism, with no effects on HIF-1α upregulation. The involvement of nitric oxide synthase (NOS) pathways in BAR3 signaling was also evaluated. The present results corroborate the hypothesis that BAR3 plays a role in hypoxia-dependent VEGF production and that its signaling could be mediated by NOS enzymes. Hence, deciphering the role of BAR3 during hypoxia may offer an additional target to counteract neovascularization.

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P2.46

Copper overload affects α-synuclein clearance mechanisms in a Parkinson's disease *in vitro* model

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Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta*. The hallmark of PD is the presence of abnormal protein aggregates called Lewy bodies (LBs) in affected neurons, mainly composed of the α-synuclein protein. Copper, an essential element, has been implicated in the aggregation of α-synuclein. This work explored the role of copper overload in the pathogenesis of PD and focused on the clearance systems of α-synuclein, such as autophagy and the ubiquitin-proteasome system (UPS), using a dopaminergic cell model derived from SH-SY5Y human neuroblastoma cells. Copper treatment increased autophagic markers Beclin-1, ULK-1 and LC3-II, altered the PI3K/AKT/mTOR pathway and impaired autophagic flux, as indicated by increased levels of LC3-II and p62 in the presence of chloroquine, a commonly used autophagy inhibitor. All these events led to the accumulation and aggregation of α-synuclein. Copper also induced an increase in polyubiquitinated proteins, indicating UPS impairment, confirmed by the use of MG132. Altogether, these data support the hypothesis that copper dyshomeostasis can compromise the clearance mechanisms of the α- synuclein protein, in particular at the UPS level, pushing the cell towards the activation of compensatory mechanisms based on autophagy.

P2.47

Bergamot essential oil (BEO) provides cytoprotection in neuronal cells exposed to heavy metals

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Bergamot essential oil (BEO) is a rich source of terpenes, flavonoids, carotenes, and coumarins whose proposed physiological activities ranging from anti-inflammatory to antioxidant, from antiproliferative to neuromodulation.
The present study was designed to explore whether BEO could attenuate heavy metal (Cd, Hg, and Pb)-induced neurotoxicity in SH-SY5Y cells, utilized as a model system for brain cells. MTT, LDH, and Calcein assays were used to examine the viability of the SH-SY5Y cells after exposure to heavy metals individually or in combination with BEO, as well as the effects of necrotic cell death, respectively. Furthermore, DCF-DA assay was performed to determine whether BEO could protect SH-SY5Y from heavy metal-induced oxidative stress.
Results allowed us to assess the capability of BEO to enhance the number of viable SH-SY5Y cells after exposure to heavy metal toxicity. Pre-treatment with BEO showed a considerable, concentration-dependent, cytoprotective effect, particularly against Cd induced toxicity. This effect was confirmed by reducing LDH release after the simultaneous cell treatment with Cd and BEO compared with Cd-treated cells. Furthermore, a significant, concentration-dependent decrease in ROS production, induced by H_2O_2 or heavy metals, was observed in the same model.
Overall, the preliminary results obtained provide information on the protective role of BEO against heavy metal-induced neurotoxicity and oxidative stress.

P2.48

Myogenic precursor cell-derived extracellular vesicles: role in sarcopenia

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The skeletal muscle aging, sarcopenia, depends on many factors, as the impaired activity of myogenic precursor cells (MPCs), the muscle stem cells progeny. Recently, the skeletal muscle was identified as a secretory organ as MPCs are able to release extracellular vesicles (EVs). EVs contain a variety of bioactive molecules as proteins and miRNA and have a pivotal role in cellular crosstalk. In detail, EVs from young and elderly MPCs culture media were collected; their physicochemical characteristics, miRNA and proteomic cargo were analyzed and a dysregulation in myomiRNAs and upregulation in cytoskeletal reorganization, actin cytoskeleton signaling and cell movement in young EVs respect to old ones were found. Then, effects of young EVs released from MPCs cultures obtained from young subjects were investigated on proliferative and regenerative features of MPCs derived from elderly ones; similarly, the effects of elderly EVs were studied on young MPCs culture. Data revealed that, due to their cargo, young EVs increased the proliferative rate and fusion index of elderly MPCs while elderly EVs reduced the proliferative rate and modulated the fusion index of young MPCs. Overall, data show that miRNAs and proteins carries by EVs affect proliferative and differentiative features of MPCs cultures depending on the age of the human donors.

P2.49

Pericyte-like differentiated adipose-derived mesenchymal stem cells improve blood-retinal barrier preservation

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The multipotent differentiation of adipose-derived mesenchymal stem cells (ASCs) has been lately investigated to develop therapeutical strategies for human pathologies where other treatments do not give satisfactory outcomes. In the present experiments, a pericyte-like differentiation was tested in order to replace the massive pericyte loss occurring in eye diseases such as diabetic retinopathy (DR), which is characterized by inflammation, oxidative stress, disruption of blood-retinal barrier (BRB). In a first step, ASC pericyte-like differentiation (P-ASC) was achieved by growing in a culture medium specifically designed for native pericytes, as assessed by the increased immunocytochemical expression of α-SMA and NG2. In the second step a coculture of P-ASCs and human retinal endothelial cells (HRECs) was carried out to mimic an in vitro BRB. Some samples were cultured in high glucose (HG) conditions (25 mM) to reproduce the diabetic environment. Results show that, even in HG conditions, a high α-SMA expression was observed, together with increased platelet-derived growth factor receptor (PDGFR) and heme oxygenase-1 levels. Altogether, results are interesting because they demonstrate P-ASC survival in HG conditions, a receptor-mediated cross-talk between ASCs and HRECs, and an increased protective effect against damages induced by reactive oxygen species. Then, P-ASCs may represent ideal candidates to develop cell-based therapies to counteract pericyte loss in DR.

P2.50

Anion exchanger 1/AE1 function is compromised in red blood cells from pre-diabetic subjects: beneficial effects of finger lime (*Citrus australasica*, Faustrime cultivar) juice extract

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Prediabetes is a high risk factor for diabetes, importantly involving oxidative stress (OS). Here, we investigated whether the anion exchange capability mediated by AE1 (*SLC4A1*) was altered in red blood cells (RBCs) from pre-diabetic volunteers. Hence, the molecular mechanisms related to OS and glycation events, which might affect AE1 ion transport activity, have been studied along with potential benefits of finger lime juice extract (*Citrus australasica*, Faustrime cultivar). RBCs from normal and pre-diabetic volunteers were incubated with 50 µg/mL juice extract for 2 h, at 25°C proving that, in the second condition, juice extract restored AE1 anion exchange capability and prevented structural rearrangements of both AE1 and α/β-spectrin. Moreover, juice extract counteracted the increased production of intracellular ROS provoking oxidation of hemoglobin to methemoglobin, reduced HbA1c levels and, finally, synergistically acted with the endogenous antioxidant system in order to suppress OS alterations in RBCs from pre-diabetic subjects. In the context of prediabetes, these findings contribute to: clarify the mechanisms related to OS and glycation events that may influence RBC homeostasis; propose finger lime juice extract as a natural antioxidant effective in counteracting RBC alterations; identify AE1 as a potential target for treatment and prevention of complications associated with a pre-diabetic condition.

P2.51

A preliminary study on the functional involvement of adrenoceptors in glioblastoma growth

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Many studies have shown that stress can promote cancer progression via catecholaminergic signaling, suggesting the targeting of adrenoceptors (ARs) as potential anti-cancer strategy. In this regard, the β3-AR antagonist SR59230A was showed to exert anti-proliferative on tumor cells. In this study, we investigated the effects of BB-14, a new SR59230A-derived compound, on human glioblastoma (GBM) U87 and U343 cells cultured either in normoxia or in hypoxia, to mimic the environment in which GBM grows. Our evidence showed that BB-14 is more cytotoxic than SR59230A both in normoxia and hypoxia. In GBM cells pretreated with norepinephrine (NE) to activate ARs, BB-14 decreased viability, while in cells pretreated with BB-14 the NE-induced increase in viability was prevented. Compared to SR59230A, BB-14 downregulated β-actin expression and blocked cell migration to a greater extent, suggesting a higher impact on cytoskeleton of GBM cells. In addition, BB-14 reduced the vascular endothelial growth factor expression more than SR59230A, indicating a greater anti-angiogenic effect. The expression profile of *α/β-ARs* analyzed by qPCR revealed that the mRNA level of *α1B-* and *α1D-ARs* was higher than that of *β1-, β2-* and *β3-ARs*. Since SR59230A was shown to also act at α1-ARs, we hypothesize that BB-14 action can involve not only β3-ARs, but also α1B/D-ARs. Our results highlight the role of a/β-ARs in GBM biology, suggesting their targeting as a promising approach to reduce GBM viability.

P2.52

Cell surface GRP78: the dark side of glucose-regulated protein 78

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A stressed ER results in UPR activation and overexpression of GRP78. During ER stress, GRP78 evades retention mechanisms and is translocated to the cell surface (csGRP78) where it functions as an autoantigen. csGRP78 is a hallmark of some cancer cells (ovarian, prostate, brain and breast cancer, myeloma, melanoma, and lymphoma), and more generally stressed cells.
In some specific cancer types, csGRP78 expression was found correlated with chemotherapy resistance, tumor recurrence and prognosis. So molecules or autoantibodies binding to csGRP78 are necessary and sufficient to induce a transduction signal and promote proliferation via activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK), p21RAS-dependent MAPK and PI3K/Akt, signaling pathways.
In agreement with our previous data on pleural mesothelioma (PM) biopsy tissues, we observed an increased basal GRP78 expression levels in PM cells, overall indicating a mild UPR as a constitutive PM condition. For this reason, we evaluated the expression of GRP78 at the cell surface and the consequent correlation with the downstream signaling.
Furthermore, we assessed the effect of the clinical-stage small molecule drug BOLD-100 on the PM cells in inducing cellular toxicity modifying the expression, localization of GRP78 and activation of downstream pathway.

P2.53

Differential cross-talk pathways of immune cells in co-culture with enterocyte-like monolayer challenged by apical vs. basolateral LPS exposure

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The monolayer of intestinal epithelial cells (IECs) exerts a guiding influence on intestinal homeostasis and on its interaction with the immune system through secretion of conditioning cytokines, thus driving innate responses primed by resident immune cells. Here, in a co-culture model we investigated the interactions between differentiated Caco-2 cells in monolayer and macrophages, for mimicking the cross-talk between enterocytes and immune cells during GI tract inflammation. The enterocyte-like monolayers were challenged with apical or basolateral LPS and then co-cultured with THP-1 derived macrophages. LPS effectively affected monolayer's permeability and levels of inflammatory mRNAs in Caco-2 monolayers. Remarkably, macrophages differentially responded based on the different directional source of LPS previously administered to Caco-2 monolayers. Basolateral sensing of LPS by Caco-2 monolayers induced specific increase of many inflammatory mRNAs including NF-kB1, IL-6 and IL-8 in macrophages, while apical sensing triggering targeted increase of IL-1β. Significantly, the analysis of immune factors secreted in the co-culture media showed that paracrine interactions between Caco-2 monolayers and macrophages are differently driven based on the basolateral vs. apical inflammation and thus involving different immune gene networks. Taken together, our results suggest a framework of interactions between IECs and immune cells depending on the "polarized" inflammatory dysregulation.

P2.54

Role of calcium ion and anionic phospholipids on the ubiquitin aggregation process

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Ubiquitin (Ub) is a small and highly conserved protein, which participates in numerous biological processes. In eukaryotic cells, the Ubiquitin-Proteasome system (UPS) is the main pathway for eliminating misfolded or damaged proteins.
The failure of the UPS towards misfolded proteins can lead to the formation of toxic oligomeric aggregates and appear to be involved in some neurodegenerative diseases including Parkinson’s, Alzheimer’s, amyotrophic lateral sclerosis, and prion diseases. The link between protein misfolding and aggregation, UPS, and neurodegenerative disorders is supported by the observation that protein aggregates within affected cells often contain Ub.
Ub stability seems to be affected by different extrinsic factors including metal ions and membrane lipids. Copper (Cu^{2+}) and zinc (Zn^{2+}) are able to bind to Ub promoting its aggregation as well as anionic lipids acting as molecular chaperones.
This study evaluated the effects of calcium ions (Ca^{2+}) and anionic phospholipids on the Ub aggregation process. The formation of Ub aggregates was assessed using dot blot and SDS-page assays and the protein's ability to permeabilize the membrane was monitored through relaxometric and single-channel current measurements.
Calcium ions and anionic phospholipids seem to favor the formation of oligomeric aggregates which incorporate into the membrane and form conductive units, suggesting a possible mechanism of Ub toxicity.

P2.55

Autoimmune mechanisms in Brugada syndrome: plasma effects on Nav1.5 activity in HEK cells and hiPSC-derived cardiomyocytes

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Brugada Syndrome (BrS) is a rare disorder related to cardiac arrhythmias and sudden death. It is mostly associated with mutations in the alpha subunit of the cardiac voltage-gated sodium channel (Nav1.5) gene (*SCN5A*). However, this does not explain the number of cardiac events occurring in individuals without *SCN5A* mutations. Also, BrS diagnosis involves invasive pharmacological tests that raise safety and ethical concerns. Giving the emerging field of autoimmune cardiac channelopathies, this study aims to investigate the effect of the BrS patients' plasma on the activity of Nav1.5 expressed in both HEK cells and hiPSCs derived cardiomyocytes (hiPS-CMs) using electrophysiological techniques. Patch-clamp experiments revealed a dose-dependent effect of one-hour incubation with BrS patient plasma. In particular, 5% plasma caused a ≈40-50% significant reduction of I_{Na} density in both cell models, while no effect was observed with control plasma. The effect was also channel specific as BrS plasma did not affect the I_{CaL} in hiPS-CMs nor the Cav3.2 current density in HEK cells, while a ≈50% of current reduction was observed on the Nav1.4 activity. These findings were independent of *SCN5A* mutations and patients' demographics, suggesting an immunopathogenic component of BrS beyond genetics and indicate a new underlying mechanism of BrS that encourage a more comprehensive diagnostic approach and comorbidity stratification.

P2.56

A study of the negative and positive dominance of heterozygous $Na_v1.5$

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In the past years, our lab characterized the impact of the cardiac $Na_v1.5$ compound heterozygosity K1578N/G1866fs identified in a young patient affected by severe arrhythmias. The heterozygous current was severely reduced in comparison to the WT condition. New experiments identified the mechanism of this phenomenon in terms of a negative dominance effect operated by the G1866fs mutation on the less severe K1578N. Interestingly, when the G1866fs mutation was expressed in heterozygosis with the WT, the current showed no alterations, suggesting a positive dominance of the WT on the mutant isoform.
$Na_v1.5$ channels are known to assemble as dimers by binding to the 14-3-3 dimeric adaptor protein and it is this interaction that provides the molecular base to interpret the dominance effects. We were able to quantitatively reproduce the experimental data by using a dimeric binomial theoretical analysis based on dominance. We next used the synthetic peptide Difopein, which removed the dominance effects by antagonizing the dimeric assembly and forced the channels into the monomeric state. These data were indeed nicely fit using a theoretical approach which did not require the concept of dominance.
Our study suggests a general framework to interpret pathological heterozygous $Na_v1.5$ conditions in terms of negative dominant behavior associated with dimeric assembly. Removal of this dominance leads to a partial phenotypic rescue which, in principle, represents a therapeutic advantage.

P2.57

The Hippo pathway as a novel regulator of dendritic cell physiology under hypoxia

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Dendritic cells (DCs) are bone marrow-derived cells that play a pivotal role in the physiology of the immune system. They act as primary sentinels and, during their lifespan, they traffic through areas characterized by different oxygen tensions. Hypoxia is a feature not only of inflamed and tumor tissues but also of lymphoid organs, including bone marrow and lymph nodes where DCs exert their main functions. Thus, the modality by which DCs adapt to hypoxia is important to guarantee their survival and, so far, for modulating the quality of the immune response. Indeed, previous reports have indicated that hypoxia influences the survival of DC cells through adaptive molecular processes involving HIF-1 to ensure their proper physiological function. However, the multitude of pathways and factors that might regulate DC survival is not fully understood. Here we report the Hippo pathway as a new potential regulatory mechanism associated with DC cell survival. We observed an association between the expression of YAP1, the master regulator of the Hippo pathway, and DCs survival marker under hypoxia, with a particular involvement of the MAPK including ERK1/2. Our data suggests that the Hippo pathway may give a novel contribution to understanding some of the molecular mechanisms involved in DC survival, with significant implications for the regulation of immune system homeostasis.

P2.58

Aging-related oxidative stress impairs cellular shape function and signaling in human erythrocytes: Açaì berry is a keystone?

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Aging is characterized by a general decline in physiological functions with reactive oxygen species (ROS) playing an important role. As functional foods rich in flavonoids are excellent candidates to counteract age-related changes, this study aimed to verify the protective role of Açaì extract in a D-galactose (D-Gal)-induced model of aging in human erythrocytes. ROS production, thiobarbituric acid reactive substances levels, oxidation of protein sulfhydryl groups, anion exchange capacity through Band 3 protein (B3p), band 3 tyrosine phosphorylation (p-Tyr), cytoskeleton-associated proteins distribution (spectrin, ankyrin, and protein 4.1), glycated hemoglobin (A1c), and erythrocytes deformability (elongation index) were analyzed after 24 h treatment with D-Gal, with or without 1 h pre-incubation with 10 µg/mL of Açaì extract.
Our results show that the Açaì extract avoids acanthocytes and leptocytes formation, prevents oxidative damage, restores alterations in B3p distribution and the rate constant for SO_4^{2-} absorption. Açaì extract also restored the increased levels of p-Tyr and Syk kinases and alterations in the distribution of spectrin, ankyrin and protein 4.1, as well as attenuated A1c levels. Finally, decrease in the deformability of erythrocyte membrane was also impaired.
These findings further contribute to clarify mechanisms of natural aging in human RBCs, and propose natural flavonoid substances for prevention of oxidative-stress-aging related.

P2.59

Selenoprotein T as a novel key player in aging-associated myocardial dysfunction

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Aging leads to the deterioration of cardiac structure and function, increasing susceptibility to heart failure. Selenoprotein T (SELENOT) is a crucial regulator of cardiogenesis and cardiac protection, but its involvement in aging-associated myocardial dysfunction is not known. Here, we designed a mimetic SELENOT peptide (PSELT), including the redox motif of the protein, and assessed its beneficial action in aged spontaneously hypertensive heart failure (SHHF) rats and in human senescent cardiomyocytes. Results showed that in the failing hearts, PSELT attenuated severe ultrastructural alterations and counteracted key mediators of cardiac aging, also preventing γH2AX upregulation (a hallmark of DNA damage). Hemodynamic studies indicated that PSELT improved contractile impairment at baseline and following ischemia/reperfusion injury in SHHF animals, reducing infarct size in both control and failing hearts. In senescent human cardiomyocytes, PSELT counteracted oxidative stress by reducing intracellular ROS generation and mitochondrial superoxide production, while also reducing SELENOT (a potential sensor of aged cardiomyocytes) and NLRP3 upregulation, both alone and in combination with inflammasome inhibitors. These effects were accompanied by the peptide's ability to mitigate the upregulation of senescent markers and relieve mitochondrial dysfunction. These findings indicate that SELENOT can represent potential therapeutic targets for counteracting cardiac senescence.

P2.60

A pilot study for preventing minerals deficiency in the adult population by functionalized Seaweed Extract lettuce

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The Seaweed extracts from Ecklonia maxima (SwE) is a biostimulant that enhances mineral concentration in vegetables. The study investigated whether supplementation with SwE lettuce influences endogenous mineral micronutrients concentration and the potential metabolic pathways involved. 55 healthy volunteers were allocated in a double-blinded manner into groups that consumed 100 grams a day of control lettuce, SwE lettuce or one tablet of iron for 4 weeks. Blood samples were collected at baseline and at the end of the trial and compared among the groups for differences in serum mineral concentrations, iron, glucose and lipid homeostasis. The consumption of SwE lettuce significantly enhanced iron serum levels. It also improved iron homeostasis by increasing transferrin saturation but did not affect transferrin and ferritin. Total cholesterol (TC) and Low-Density Lipoprotein (LDL) were reduced.The consumption of SwE lettuce did not affect glucose homesoasis. Iron supplements increased serum iron and transferrin saturation, TC and LDL similar to SwE lettuce but with side effects like diarrhea or constipation. The study showed that consumption of SwE lettuce increases iron concentration within physiological range and ameliorates iron homeostasis.

P2.61

Physiological response of human osteoprogenitor cells on 3D-printed polymeric scaffolds for bone tissue regeneration

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Large bone defect treatments have always represented an important challenge in clinical practice and created a large demand for more efficacious regenerative approaches. The bone tissue engineering (BTE) strategy offers a new alternative to conventional bone grafts, addressing all clinical needs. In last decades, scaffolds based on naturally derived polymers, such as Gellan gum (GG), have emerged as a novel class of biomaterials for BTE applications due to their inherent physical and chemical properties and good biocompatibility. Furthermore, scaffold matrix is often combined with hydroxyapatite (HAp) and bioactive agents to improve bone formation and repair. In this study we evaluated the biological response of osteoprogenitor cells grown on different types of 3D-printed methacrylated GG (GGMA) scaffolds (pure GGMA, GGMA/Hap, GGMA/BSF-Eumelanin), in terms of biocompatibility, osteoconductivity, and osteoinductivity. Our results highlighted that GGMA/HAp and GGMA/BSF-Eumelanin triggered a different, time-dependent, physiological response in the osteoprogenitor cells cultured on them, compared to pure GGMA. Specifically, both the functionalized scaffolds were able to promote cell adhesion, viability, proliferation, and osteogenic differentiation. These findings suggest that the combination of osteoprogenitor cells with functionalized 3D-printed polymeric scaffolds represents a promising strategy in the field of orthopaedic surgery for the treatment of large bone defects.

P2.62

The effect of cigarette smoking on the cardiorespiratory and metabolic kinetics in young physically active males

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Background: Evidence on cigarette smoking (CS) effects on exercise capacity in young smokers (SM) with high fitness level at the early stage of CS history is limited and controversial. This study evaluated the cardiorespiratory and metabolic kinetics before and after moderate exercise in young, healthy and physically active SM.
Methods: Ten SM (age: 21±2 yr., body mass: 78±5 kg; 11±5 cigarette/day for 6±2 yr.; mean±SD) and 12 non-smokers (CTRL; age: 24±3 yr., body mass: 79±10 kg) were enrolled. After the maximum pulmonary oxygen uptake ($V'O_{2max}$) assessment on a cycle ergometer, participants performed four transitions to a 6-min square-wave exercise (90% of gas exchange threshold). Expiratory ventilation (V'E), $V'O_2$ and heart rate (f_H) response was recorded and fitted by a mono-exponential function to assess the time constant (τ) in both on- and off- phase.
Results: At peak exercise, SM exhibited lower V'O2 (-6%; P=0.05), mechanical power (-10%; P=0.008) and V'E (-9%; P=0.013). During the on-phase of the square-wave exercise, SM reported longer τ in $V'O_2$ (+33%; P=0.010), V'E (+45%; P=0.01) and f_H (+20%; P=0.015). During the off-phase, the longer τ was still present in $V'O_2$ (+18%; P=0.008) and f_H (+33%; P=0.018).
Conclusions: These findings are compatible with an early CS-related impairment of the cardiorespiratory and metabolic function even at the moderate exercise in young, healthy individuals with relatively short CS history.

P2.63

Exploiting partial reprogramming to rejuvenate Drosophila melanogaster: a novel approach to assess the role of intestinal stem cells on age-dependent degradation of the intestinal barrier

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Partial reprogramming refers to the process of inducing temporary, controlled expression of reprogramming factors to rejuvenate cells without fully reverting them to a pluripotent state. This technique uses the Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) to induce reversal of age-related hallmarks and improvement of cellular function, a state sometimes referred to as rejuvenation. The lack of suitable models for partial reprogramming of an entire organism has limited the research in this field. The aim of this study is to create a genetically engineered Drosophila melanogaster that expresses human OSKM in GFP-expressing stem cells using a temperature-sensitive inducible system for pulsatile reprogramming. In adult flies, stem cells accumulate mostly within the intestine where they preserve gut functions against age-dependent degradation of the intestinal barrier. We showed that adult induction for 12h (starting from larvae stage) appears to be safe and viable. On the other hand, early embryonic pulsatile induction proved to be lethal due to the abundance of stem cells, improper cell differentiation and tissue formation. In conclusion, we produced a viable genetically engineered model of Drosophila melanogaster to investigate partial reprogramming. The next phase of our study will use this model to investigate the effects of gut cell rejuvenation and its role in ageing and health.

P2.64

Diet-induced impairment of skeletal muscle and adipose tissue metabolic homeostasis and its prevention by probiotic administration

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Western dietary pattern is one of the main contributors to the increased risk of obesity and chronic diseases, through oxidative stress and inflammation in the most relevant metabolic organs, such as skeletal muscle. Indeed, the chronic exposure to high levels of dietary fatty acids can increase the amount of intramyocellular lipids in skeletal muscle, altering glucose homeostasis and contributing to reductions in muscle protein synthesis and mitochondrial oxidative capacity. Probiotics administration is a promising approach as preventive strategies to attenuate Western diet induced metabolic damage. Therefore, we verified the potential beneficial effect of *Limosillactobacillus Reuterii* DSM 17938 on the inflammatory state and oxidative balance in the skeletal muscle and adipose tissue in adult rats fed a western diet (8wks), focusing on the role of skeletal muscle mitochondria. *Limosillactobacillus Reuterii* DSM 17938 administration protects the skeletal muscle from mitochondrial dysfunction and oxidative stress, preventing the establishment of inflammation and insulin resistance. In addition, it exerts a beneficial effect on the body composition, favoring the deposition of protein mass and preventing adipose tissue hypertropy and inflammation. These results open the possibility for the use of this probiotic as a therapeutic approach for nutrition-related diseases.

P2.65

Impact of intraluminal valves on hydraulic resistance and lymph flow in lymphatic collectors

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Collecting lymphatic vessels are endowed with intraluminal unidirectional valves essential for propelling lymph from interstitial and serosal cavities upstream to the venous blood circulation, playing a pivotal role in preventing lymph backflow in the low-pressure lymphatic system. These valves, despite their importance, introduce unavoidable lumen restrictions, potentially increasing hydraulic resistance and elevating the energy loss of lymph as it moves through the vessel network.
To investigate these effects, we measured lymph flow velocity inside unobstructed vessel segments and during lymph transit through the adjacent intraluminal valve. We also used numerical simulations to compute the supposed hydraulic resistance, pressure drop, and head loss (defined as energy loss due to frictional forces) as lymph flows through the intraluminal valve. Our measurements of intraluminal hydraulic pressure at sites around these valves revealed that, although the presence of valves increases hydraulic resistance, the supposed pressure drop is significantly lesser (about one order of magnitude) than the measured intraluminal pressure swing induced by spontaneous contractions of the lymphatic muscle. Preliminary data thus indicate that the head loss introduced by intraluminal valves does not result in a significant restriction to lymph propulsion.

P2.66

Do humans perceive the odor of fatty acids by an orthonasal pathway?

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Smell strongly influences food choice and its hedonic evaluation, playing an important role in the intake of nutrients, in the control of meal size and in the food nutritional composition even before it is consumed. Smell contributes to the perception of fatty acids (FAs) through the retronasal pathway, while their olfactory perception through the orthonasal pathway is still a topic of discussion. The olfactory function of 28 healthy subjects (15 F, 13 M, aged 22-45 y) was assessed by means of the “Sniffin’ Sticks” Test. The ability to perceive the odor of palmitic (PA), oleic (OA) and linoleic (LA) acids was evaluated with the coupled Gas Chromatography-Olfactometric (GC-O) technique. The number of participants who smelled PA was significantly lower than those who smelled OA and LA. Participants classified as normosmic, reported perceiving the odor of FAs with higher intensity than hyposmic ones. The perception intensity of FAs odor reported by the participants showed a decreasing order: LA>OA>PA for normosmic individuals and LA=OA>PA for hyposmic ones. All participants showed the following decreasing order of perception threshold for FAs: PA>OA=LA. Results confirm the human orthonasal ability to perceive the odor of FAs and show that the intensity of perception increases as the lipophilicity of FAs decreases; consequently, the olfactory perception threshold also decreases.

P2.67

Antioxidant role of bovine colostrum in a colitis murine model

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Bovine colostrum (BC), the initial milk produced by cows after giving birth, has revealed significant potential in helping various health conditions, particularly in diseases of the gastrointestinal tract such as inflammatory bowel disease, including colitis and Crohn's disease. Inflammatory bowel diseases are characterized by elevated oxidative stress, leading to tissue damage and exacerbated symptoms. BC is renowned for its rich composition of macronutrients, micronutrients and bioactive compounds, that boost the immune system and make BC an excellent health supplement. The aim of this study was to explore the potential antioxidant activity of BC supplementation in a mouse model of colitis induced by trinitrobenzene sulfonic acid. The study was carried out by analyzing the immunohistochemical expression of primary antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase in the colon. The results of this investigation have the potential to unveil BC as a natural strategy for the management of oxidative stress-related gastrointestinal disorders.

P3.1

Morphofunctional study of serotonergic neurons in the adult human cerebellum

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In recent studies a cerebellar role in serotonin (5-HT) related functions and disorders have been highlighted. The cerebellum receives a serotonergic innervation from the reticular formation. Data of an intrinsic cerebellar serotonergic system are lacking, and the mechanism by means 5-HT affects the neuronal firing frequency are still poorly understood. Although, in the cerebellum changes in the intrinsic electrical properties of the neurons upon application of 5-HT have also been reported. Therefore, the aim of this study is to evaluate in the human cerebellum the existence of serotonergic neurons. The study was carried out on postmortem fragments of the human cerebellum, fixed in a picric acid-aldehyde solution, embedded in paraffin, cut into 5µm sections and subjected to light microscopic immunohistochemistry with a rabbit polyclonal antibody for 5-HT. In the cerebellar cortex the 5-HT immunoreactivity (ir) in basket neurons, Purkinje neurons and in some non-traditional large neurons have been observed. In the dentate nucleus the 5-HT ir in perivascular neurons, associative and projective neuron types have been detected. Therefore, in the human cerebellum a role of 5-HT in neuromodulation mechanisms and in vasoactive functions is suggested. Moreover, a cerebellar role in 5-HT-related brain disorders, and perhaps a target for pharmacological and non-pharmacological innovative therapies is also suggested.

P3.2

The novel E83Q SNCA mutation drives specific protein handling, interactors and disease-phenotypes

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The novel E83Q SNCA mutation was shown to cause dementia with Lewy bodies with cortical areas presenting severe Lewy pathology and gliosis. A previous study showed that when hippocampal or cortical primary neurons are treated with PFFs derived from human mutant (alpha-synuclein) aSyn, we observed a greater morphological diversity. To evaluate whether seeds carrying the human E83Q mutation were able to trigger different pathological features, we inoculated human WT or human E83Q PFFs in the dorsolateral striatum of adult C57BL6/J mice and followed the progression of the disease at different timepoints post-injection. Furthermore, using an expanded toolbox of antibodies, we evaluated aSyn pathology and post-translational modifications in postmortem human tissue of th eonly patient carrying this mutation. In general, we saw an accelerated and more prominent aSyn pathology in hE83Q-injected mice, with brain areas reminiscent of frontotemporal dementia. In the postmortem tissue, aggregates displayed specific PTMs that points towards a different protein handling. Finally, proteomics displayed region-specific interactors present in insoluble aSyn aggregates

P3.3

Changes in pupil responses precede behavioral adjustment in a rewards stop-signal task

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Action control is regulated by the rewards that may be gained or lost as a result of the choice made. In integrative physiology, this process has often been studied with a focus on the somatic nervous system, overlooking the role of the autonomic nervous system. To bridge this gap, we recorded an index of autonomic activity (pupillary dilation or PD) in subjects performing a Reward Stop Signal Task. This task assesses a subject's ability to control actions by two types of trials: Go trials, where subjects must respond as quickly as possible to a Go signal, and Stop trials, where a Stop signal is presented following the Go signal, requiring the subjects to halt. We manipulated the rewards by presenting a Cue before each trial: G+, where the reward was greater for Go trials than for Stop trials; S+, where the opposite reward schedule was used; and N, where the same reward was given for both. Rewards were in the form of points gained, and the goal for each subject was to accumulate as many points as possible to rank first among participants. We found that behavior reflected the different contexts: subjects were faster to respond and more likely to make errors in Stop trials in the G+ condition compared to the S+ condition. This behavior was anticipated by changes in PD following the Cue presentation. PD was higher in the G+ condition compared to the S+ condition. These findings show that somatic and autonomic activities are integrated to achieve specific behavioral goals.

P3.4

Optimized sensorimotor activation enhances real-time visuomotor coordination in goal-directed aiming

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To better understand how an optimized focal muscle vibration (fmv) affects motor control, we investigate the fmv impact on upper limb aiming movements guided by visuomotor coordination or internal control.
First, we applied the fmv on deltoid muscles that assist with shoulder movements in healthy individuals. Then, we evaluated the immediate and one-week-after effect on upper limb aiming movements prepared and executed under various visual conditions. Fmv was optimized for spatial-temporal stimulus propriety, duration, number of applications, and concomitant muscle contractions. We used a 3D optoelectronic motion capture system to assess movement accuracy and kinematics.
We found that fmv improved mean speed and movement smoothness and ameliorated accuracy only when tasks were planned and executed with visual feedback. The improvement began immediately and increased one week after fmv.
Thus, fmv may impact visuomotor coordination over time without affecting internal control of aiming movement. Data indicate that fmv aftereffects do not reflect modifications in kinesthetic information processing. Instead, fmv could improve how the brain uses proprioceptive information to translate a visuospatial plan into motor commands, enhancing muscle engagement when initiating movements through real-time visual pathway route activation. Results suggest a possible use of fmv in developing intervention strategies to facilitate the effectiveness of motor skills acquisition.

P3.5

Evidence for joint action awareness in macaque monkeys

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Cooperation is considered an evolutionary advantage for humans. Our aim is to study the neural processes underlying interindividual motor coordination using an animal model. Previous studies in our lab demonstrated that macaques are able to adjust their behavior to perform joint actions (JA) in a controlled lab setting, consisting in pairs of animals sitting next to each other in front of a screen, and guiding visual cursors, either individually or together, through isometric joysticks. The question here is to understand if macaques are aware of acting together in this abstract video-game context, to assess the suitability of our model to explore the neural bases of motor interactions. Video-recordings of animal behavior were used to analyse spontaneous head rotations (SHR) and their time of occurrence relative to the different task phases, considering SHR of each animal toward its partner as a sign of animal awareness of acting with its mate. We found that SHR were more frequent during joint-than individual actions, and further increasing their rate in errors trials. Dyadic actions, due to the cost of coordination, result in a higher error rate as compared to solipsistic actions, and they require constant monitoring of own and other's behavior to optimize joint performance. These results provide evidence for awareness of acting together in macaque, and can guide our work on the neurophysiology of action monitoring and coordination, during social interactions.

P3.6

Effect of early life stress on cerebellar development

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Exposure to stressful events during the first days of life increases the vulnerability to neuropsychiatric diseases. Indeed, normal maternal behavior ensures a quiescent stress response in the pups, characterized by relative unresponsiveness to external stressors and low basal ACTH and corticosteroid levels, thus having a protective role in the developing brain. Our study aimed to examine in mice the effects of maternal separation (MS), a rodent model of early life stress, on the cerebellum, in which neurogenesis and synaptogenesis occur extensively after birth. Structural analysis revealed that MS causes an alteration of Purkinje cell dendritic arborization associated with a significant change in the density of excitatory climbing fiber synapses. Using quantitative PCR and western blot analysis, we found that MS caused a substantial alteration of BDNF signaling in the cerebellum, which may be responsible for synaptic structural changes. Genome-wide DNA methylation profiling revealed epigenetic signatures in the cerebellum of MS offspring. These results suggest that early life stress may alter the proper development of the cerebellar neural circuits during a critical and sensitive period.

P3.7

Evidence of EEG correlates of memory-based decision-making during a transitive inference task

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Making decisions based on previously acquired knowledge is fundamental for optimizing interactions with a complex environment. The transitive inference task is an experimental paradigm developed for studying the cognitive and behavioral correlates of this ability. It first requires learning the reciprocal hierarchy between the adjacent items of a rank-ordered set, like the series A>B>C>D>E>F, and then indicating, in a test phase, the ordinal relationship between all possible pair combinations (e.g.,C?F). When performing this task, the accuracy in determining whether a given item is higher or lower in rank than another depends on their rank differences. In fact, it is easier to compare items with a larger rank difference than smaller ones. This phenomenon highlights a symbolic distance effect (SDE), which is thought to reflect a spatial-like mental representation of the item's rank, arranged linearly in working memory. Monkey neurophysiology studies have found a correlation between SDE and the activity of neurons in both the parietal and prefrontal areas. Using EEG with human participants, we found modulation of ERP components involved in visual attention from corresponding brain areas, providing a functional link, from microscopic to macroscopic scales, emphasizing the role of these brain regions in this cognitive function.

P3.8

Long-term plasticity in cerebellar microcircuit: preliminary findings and the role of clozapine

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The cerebellum, traditionally known to control movement and motor coordination, is gaining importance for its association with cognitive, and emotional processes with long-term plasticity central to these functions. Our in vitro studies on rodent acute cerebellar slices, utilizing the 3Brain microchip technology and custom-made software for analysis, have revealed that long-term depression is a dominant form of plasticity at the mossy fibre/granule cell synapses, influenced by inhibitory mechanisms. Additionally, alterations in the Purkinje cell firing rate and pattern were observed. While the effect of clozapine, one of the effective atypical antipsychotic drugs that are widely used to treat drug-resistant schizophrenic patients, in the forebrain and limbic regions is well-studied in schizophrenia, its influence on the cerebellum, an emerging area of interest in the cognitive impairments associated with this disorder, remains largely unexplored. To elaborate on these findings, ongoing experiments are assessing the impact of clozapine on cerebellar microcircuit plasticity and the underlying inhibitory-excitatory dynamics. Investigating how clozapine affects cerebellar microcircuit and long-term plasticity could reveal novel aspects of its action in the cerebellum, offering valuable insights into its therapeutic application in neurological disorders.

P3.9

Monocular delay during active vision shifts ocular dominance

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Delaying information in one eye by 330ms for 1h during an active visuomotor coordination task (building a tower from blocks) is sufficient to transiently shift ocular dominance in favor of the delayed eye (Steinwurzel et al, CurrBio 2023). Instead, passively viewing a third person performing the same task does not affect vision, despite the same visual delay. This leaves open the possibility that the sight of one's own movements (egocentric perspective) is critical for monocular delay to shift ocular dominance.
13 participants performed three experiments that measured ocular dominance balance before and after 1h altered-reality visual stimulation. The first experiment was a replication, with participants actively performing a tower-building task in a VR setup that delayed signals to the dominant eye by 330 ms. Like in our previous study, this transiently shifted ocular dominance in favor of the delayed eye. In the second experiment, participants passively watched the video recorded during the first experiment. This did not shift ocular dominance, indicating that active engagement in the task is necessary. The third condition replayed the video in one eye and blanked the other, reproducing a standard monocular deprivation condition; the effect was statistically indistinguishable from the first experiment with active task performance.
We conclude that an active visuo-motor active task is necessary for delayed visual input to induce ocular dominance plasticity.

P3.10

The epilepsy gene *TBC1D24* regulates intra-organellar pH homeostasis in neurons and synapses

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TBC1D24 is a gene mutated in a broad spectrum of neurodevelopmental disorders, from mild epilepsy to severe epileptic encephalopathy. TBC1D24 protein regulates neuronal development and synaptic function; yet the molecular mechanisms mediating these complex roles and their relation to brain dysfunction are largely unknown. By immunoprecipitation and cell staining experiments, we revealed the interaction between Tbc1d24 and the cytosolic domain of vacuolar-ATPase (v-ATPase) in the brain. Using *Tbc1d24* knockout neurons, we found that loss of Tbc1d24 leads to a decrease in the assembled and active v-ATPase complex with parallel impairment of intracellular organelles acidification. This phenotype was accompanied by defects in the autophagic clearance. At synaptic level, Tbc1d24 loss resulted in a reduction in the number of synaptic vesicles with the accumulation of endosomal-like structures, dysregulated synaptic autophagy and altered synaptic vesicle acidification. In IPSC-derived neurons from TBC1D24 patient we revealed a significant loss in TBC1D24 expression, alteration of intra-organellar pH and altered electrophysiological phenotype characterized by the appearance of bursts with increased duration and altered timing.
We propose a novel function for TBC1D24 as a regulator of V-ATPase activity in neurons and suggest pH homeostasis dysregulation as a key cellular mechanism that underpins the synaptic defects and pathogenesis in *TBC1D24*-related disorders.

P3.11

Higher efficacy of 1,3-butanediol as a neuroprotective strategy in hippocampus of healthy rats compared to calorie restriction

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Ketogenic diet has a broad therapeutic potential but presents practical limitations due to its low compliance. Therefore, several dietary supplements have been developed to induce exogenous ketosis, and the ketogenic effect of the alcohol 1,3-butanediol (BD) was demonstrated. In order to assess whether BD influences brain homeostasis in physiological conditions, we investigated the impact of a short-term BD administration in the hippocampus of healthy rats in comparison to that observed in rats undergoing a restricted dietary regimen (RD), isoenergetic with BD group.
ROS content and the extent of oxidative damage to proteins were lower in BD and RD than in control rats (C group). Decreased amount of lipoperoxides, lower susceptibility to oxidative insult, higher amounts of the enzymes superoxide dismutase-2, glutathione reductase and glutathione peroxidase (GPx), and increased GPx activity were observed in BD rats, but not in RD rats. Accordingly, reduced pro-inflammatory signaling pathway and glial activation were revealed in BD. In addition, BD administration attenuated endoplasmic reticulum stress, reduced the activation of autophagic response, and was associated with an increase of BDNF and synaptic markers. Our results highlight that BD plays a neuroprotective role in healthy conditions, independently from energy intake reduction associated with its assumption, thus representing an effective tool to support brain function without implementing nutritional interventions.

P3.12

Synthetic torpor enhances resistance against radiation: transcriptome profiling of radioprotected livers

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Torpor is a peculiar physiological state characterized by a reversible depression in metabolism and body temperature, adopted by some animals to survive challenging conditions. While in torpor, animals engage still unknown adaptive responses boosting their resilience to radiation. To exploit such radioprotection translationally, we investigated the effects of radiation exposure in rats induced in synthetic torpor (ST), a state resembling natural torpor, but induced in non-hibernators through the pharmacological inhibition of Raphe Pallidus, a key nucleus of the cold defense pathway.
Sixteen male rats were implanted under general anaesthesia with a microcannula targeting RPa and a thermistor to record brain temperature (Tb). The experiment lasted 8 hours, during which rats received hourly injections of either the $GABA_A$ agonist muscimol to induce ST (hypothermia, Tb~22°C) or vehicle (normothermia, Tb~37°C). After the fourth injection, half of either hypothermic or normothermic rats were exposed to 3 Gy of total body X-rays, while the remaining were sham-irradiated. After the eighth injection, liver samples were collected for histological and RNA-sequencing analysis.
At histological level, ST enhanced liver resistance to radiation. Transcriptome profiling showed early responses to radiation exposure in ST animals, along with a discrete group of genes potentially crucial in ST-mediated radioprotection mechanisms.

P3.13

Altered synaptic plasticity in neurodevelopmental diseases: effect of 5-HT7R stimulation

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Synaptic plasticity involves adjustments of neuronal circuits, including variations in the number of synapses and remodeling of preexisting synaptic connections. Nerve endings ability to synthesize proteins independently of the cell body enable them to respond appropriately and quickly to the local stimuli. Interestingly, several neurodevelopmental diseases (NDDs) are associated with dysfunctions at synaptic levels.
Aim of this work was to investigate the molecular mechanisms underlying altered synaptic plasticity in different NDDs to unveil possible common therapeutic targets. We isolated synaptosomes from two NDDs animal models: BTBR, as mice model for autism spectrum disorder, and Angelman syndrome (AS) mice model. Our results revealed that synaptic protein synthesis is altered in both BTBR and AS synaptosomes.
Interestingly, serotonin receptor 7 (5-HT7R) is involved in synaptic plasticity and its deregulation has been associated with NDDs. When we stimulated 5-HT7R with the selective agonist LP-211, we observed an enhanced synaptic protein synthesis in both BTBR and AS synaptosomes. Moreover, 5-HT7R stimulation rescued the altered dendritic spines density in AS primary mouse hippocampal neurons.
In conclusion, 5-HT7R emerged as a new therapeutic target for the NDDs characterized by altered synaptic plasticity.

P3.14

Angelman syndrome: a study on neuronal cultures derived from patients' iPS cells

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The Angelman syndrome is a neurodevelopmental disorder due to mutations in UBE3A gene, which is a member of the ubiquitin-proteasome system and is involved in the degradation and regulation of post-traditional proteins. In humans and rodents, UBE3A/Ube3a transcript is maternally imprinted in several brain regions, allowing the creation of a mouse model of Angelman syndrome (Jiang et al., 1998), which is widely used to study neurodevelopmental alterations and neuropathological features. For this background, to establish a human model of the disease, we started from 5 different hiPS cell lines from Angelman patients, each of which carrying a different genetic mutation, and 3 hiPS cell lines from healthy patients as controls. After verifying stemness, we differentiated them into Neural Progenitor Cells (NPCs), passing through Embryonic Bodies (EBs) formation, to further analyse their proliferation and differentiation capability. During all these steps, WB analysis have being performed to monitor UB3A enzyme during neural differentiation, whose expression lack in neurons in Angelman syndrome, but is not altered in other brain cells. In parallel, since an accumulation of proteins - due to a block of autophagy - has been reported in the brain of the mouse model of Angelman syndrome with consequences on cognitive processes, NPCs have being differentiated into cortical mature neurons and analyzed for their morphology, action potential transmission and autophagy flow.

P3.15

Synaptic neurofilaments changes during aging and the effect of environmental enrichment

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Neurofilaments (NFs), neuron-specific cytoskeletal proteins, are heteropolymers composed of four subunits: NFL, NFM, NFH, and αINTX. The synaptic population (SNFs) differs from the axonal subgroup and subserves unique functions, such as the maintenance of synaptic integrity and the modulation of neurotransmission. During aging, synapses undergo several changes, contributing to the worsening of cognitive performances and the so-called age-associated memory impairment. However, the role of senescent SNFs has never been investigated.
Environmental enrichment (EE) is a housing protocol which encourages novelty and explorative behaviours, along with interactions, cognitive stimulation and physical activity. Here, by applying the EE paradigm, we aim to understand the potential role of SNFs in age-dependent plasticity.
By employing biochemical analyses, confocal microscopy and behavioural tests, we found a cortical and hippocampal modulation of synaptic NFs expression pattern during aging and in response to the EE protocol, with synaptic NFL levels correlating with cognitive performances. These findings highlight the role of a potentially novel target, whose functional arrangement might be influenced by EE.

P3.16

Motion of the touched surface alters the trajectory of hand movements in a reaching task

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The brain has the remarkable ability to adapt motor behaviour when facing a vast range of changes in the surrounding environment and in one's own body. Reaching movements are well-studied instances of this adaptability, where rapid adaptation occurs in response to a variety of changing environment. We studied the role of touch in a reaching task by challenging a fundamental prior assumption—that self-motion of inanimate objects is unlikely upon contact. Participants slid their fingertips across a robotic interface, with their hand hidden from sight. Unbeknownst to the participants, the robotic interface remained static, followed hand movement, or moved in opposition to it. To this end, the control algorithm updated the position of the robot as a function of the displacement of the hand and of an arbitrary gain, whose value was set by the experimenter. We considered two hypotheses. Either participants were able to account for surface motion or, if the stationarity assumption held, they would integrate the biased tactile cues and proprioception. Motor errors consistent with the latter hypothesis were observed. Across multiple experiments, the role of visual feedback, tactile sensitivity, friction, and motor adaptation was also investigated. Our study carries profound implications for the interplay of prior knowledge and multisensory processing in motor control of the upper limb.

P3.17

Neurons derived from sporadic Alzheimer’s disease hiPSCs reveal morphological and functional alterations associated with downregulation of LIMK1-cofilin axis

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Human-induced pluripotent stem cells (hiPSCs) have emerged as a valuable tool to study neurodegenerative disorders, including nongenetic forms with unknown etiology, such as sporadic Alzheimer's disease (sAD). In the present study, we reprogrammed fibroblasts from sAD patients and age-matched controls (Ctrl) to generate and characterize hiPSCs and derived neurons. Both sAD- and Ctrl- hiPSCs exhibited similar stemness features and genomic stability. However, they differed in neuronal differentiation and function. sAD-derived neurons (sAD-hN) displayed higher levels of AD-related proteins, i.e., Aβ and phosphorylated tau. Patch-clamp recordings showed decreased spontaneous synaptic activity in sAD-hN compared to Ctrl and a higher firing rate in response to current injections in a subpopulation of sAD-hN. Molecular analyses revealed a significant reduction in synaptic proteins such as Synapsin-1, Synaptophysin, PSD95, and GluA1 in sAD-hN compared to Ctrl. Live-imaging experiments showed a significant decrease in neurite lengths paralleled with a decline in LIMK1 phosphorylation in sAD-hN, suggesting disruptions in cytoskeletal dynamics crucial for neuronal morphology and function. Interestingly, we also observed decreased LIMK1 expression in the AD mouse model, 3×Tg-AD. Finally, we generated an AAV chemogenetic LIMK1 to boost the LIMK1-cofilin axis, which could represent a potential therapeutic strategy to preserve cytoskeletal dynamics and neuronal function in sAD neurons.

P3.18

Different neuronal cell types in the primate prefrontal cortex differently contribute to the rank-based information encoding

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Within the cortex cytoarchitecture, pyramidal neurons and interneurons have been shown to subserve different circuit operations while encoding the received information. Here, we studied the specific role of the functional equivalent of these subclasses in neurons recorded from the prefrontal cortex (PFC) of macaque monkeys performing a highly demanding Transitive Inference (TI) task. In a sample of 158 PFC neurons, we classified the putative pyramidal (67%) and interneurons (25%). The TI task required the animals to learn the relationship between adjacent items of a ranked series (A>B>C>D>E>F) in every session, and then during a consequent test phase, use the learned relations to infer the relationship in non-adjacent novel item pairs (e.g., B-E). Earlier works have demonstrated that PFC activity is modulated by the Symbolic distance (SDist), defined as the difference between the item ranks constituting the compared pairs. We found that interneuron activity was significantly modulated by SDist, while this encoding was absent in the pyramidal subpopulation. Moreover, the performance during the test phase showed a continuous effect of learning corresponding to higher accuracy during the second half of the task. This effect was reflected in all neurons by a change in delay activity, however, interneurons showed a significantly higher rate of activity modulation than pyramidal neurons. These results suggest a higher involvement of PFC interneurons in encoding the task variables.

P3.19

Synaptic adaptations in the Nucleus Accumbens core and incubation of Meth craving after social choice-induced voluntary abstinence

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Social choice-based voluntary (SV) abstinence from methamphetamine (Meth) in rats attenuates cue-induced drug craving at late abstinence (incubation of craving) relative to rats subjected to a forced abstinence from Meth. A key underlying factor fueling cue-induced craving in rats under forced abstinence is the build-up of calcium-permeable AMPA receptors (CP-AMPARs) in medium-spiny neurons (MSN) of the nucleus accumbens (NAc) core. We hypothesized that SV abstinence would prevent CP-AMPAR accumulation in NAc core MSNs. Surprisingly, our observations revealed a similar accumulation of CP-AMPARs in both forced and SV and abstinent rats. This observation suggests that other synaptic adjustments occur within the NAc of SV abstinent rats that likely contribute to the reduced cue-induced Meth craving observed. To delve deeper, we investigated alterations in excitatory and inhibitory post-synaptic currents in NAc MSNs from rats under Forced or SV abstinence.

P3.20

The remarkable impact of opuntia ficus indica fruit administration on metabolic syndrome: correlations between cognitive functions and oxidative stress in the high-fat, diet-fed rat model

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Opuntia ficus-indica (OFI) is well-documented for its health-promoting attributes, though its fruits (OFIF) remains understudied. Metabolic Syndrome (MetS), an oxy-inflammatory disease, is known to be strictly linked with cognitive impairment. In this study, we employed a High-Fat Diet (HFD)-induced MetS rat model to investigate the efficacy of OFIF supplementation in mitigating cognitive and affective deficits associated with MetS. Following 8 weeks of HFD to induce MetS, rats received OFIF oral supplementation for 4 weeks to evaluate cognitive and affective modifications using behavioral paradigms, i.e. open field, burrowing, white-dark box, novelty-suppressed feeding, and object recognition tests. Our investigation extended to biochemical measures of oxidative stress together with leptin homeostasis. In detail, we assessed systemic antioxidant defenses and oxidants, alongside brain malondialdehyde levels. These markers were analyzed, with a particular focus on circulating leptin levels, via cross-correlation. Our data revealed that OFIF modulation of leptin positively correlates with systemic and brain oxidative stress and with markers of increased anxiety-like behavior. On the other hand, leptin levels reduced by OFIF are associated with improved antioxidant barriers and declarative memory. This study underscores OFIF potential in addressing MetS-associated cognitive repercussions, offering insights into its mechanisms and implications for future strategies.

P3.21

Blood biomarkers predicting stress vulnerability

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Stressful situations can trigger psychiatric disorders and mental health conditions. However, not all stress-exposed individuals develop mood disorders, thereby revealing the existence of two sub-populations composed of stress-resilient and stress-vulnerable subjects. Aim of this work was to identify blood biomarkers allowing to predict whether an individual will develop mood disorders in response to stress. To this end, we collected blood from both male and female naïve C57Bl/6 mice prior to exposing them to a short Unpredictable Chronic Mild Stress protocol. Mice were then clustered in vulnerable and resilient subgroups based on their performance when subjected to behavioral tests. Whole blood metabolome analyses from samples collected prior to stress exposure revealed mice clustering based on sugar metabolites, and especially myo-inositol, that nicely correlated with behavioral clustering. Given the role of inositol in regulating astrocytic activity and the involvement of glia in depression, we then investigated the content of circulating astrocytic-derived extracellular vesicles (ADEVs) as a proxy for astrocytic function and as potential source of biomarkers predictive of stress vulnerability. Indeed, transcriptomic analysis of ADEV cargo revealed differences in microRNAs associated with depression and inositol signaling. Collectively, our data suggest that poorly invasive blood testing may reveal a molecular fingerprint predictive of stress response.

P3.22

Chronic social defeat stress increases prefrontal cortex excitability and Gsk3 activation in mice

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The chronic social defeat stress (CSDS) protocol is considered as a physiological correlate of depression in mice, as it induces social avoidance, despair behavior and anhedonia. The medial prefrontal cortex (mPFC) is a main neural center of the network controlling mood and behavioral responses to stress. However, the effects of chronic stress on mPFC neuronal activity and the underlying molecular mechanisms are not fully understood. We recently reported that the blood levels of the kinase Gsk3 are different in depressed patients with bipolar disorder compared to major depression and to healthy subjects. The aim of this research was to identify how chronic stress affects neuronal excitability in the mPFC and to study the mechanistic role of Gsk3 in such responses. Pyramidal neurons were patch-clamp recorded in slices of mPFC. The degree of Gsk3 activation was estimated by western blot of total and phosphorylated forms Gsk3. Following CSDS, mPFC pyramidal neurons showed a higher number of action potentials in response to depolarizing current, indicating an enhanced excitability. Following CSDS, Gsk3 phosphorylation was significantly reduced in mPFC, corresponding to higher enzymatic activity, in line with the increased neuronal excitability. Future studies will address the causal relationship between Gsk3 hyperactivity and increased neuronal excitability in response to CSDS.

P3.23

Connectional organization reflect functional specificities of the different sectors of the macaque ventrolateral prefrontal cortex

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A recent study demonstrated that the monkey ventrolateral prefrontal cortex (VLPFC) processes contextual information relevant for action guidance (Rozzi et al. 2023). A series of neuroanatomical studies indicate that this region is characterized by a rostro-caudal organization, in which caudal and middle areas are mainly connected to oculomotor and skeletomotor territories, respectively, while rostral ones mainly show intra-prefrontal connectivity (Gerbella et al. 2017). To verify whether these connectional differences relate to corresponding functional specificities, we employed hierarchical clustering and decoding analyses to investigate neural activity recorded in anatomically defined VLPFC sectors, during the execution of a Visuo-Motor task in which monkeys were require to perform two conditions, based on different instruction cues and objects: execute or withhold grasping actions.
The results showed that: 1) caudal 12r and caudal 46v neurons are involved in coding cues and objects; 2) middle 46v neurons appear to be mainly involved in the preparation and implementation of the behavioral response, especially in the execution condition; 3) middle12r neurons code cues, objects and behavioral response, in both conditions. Altogether, our findings suggest that the various VLPFC areas are differentially involved in exploiting the various types of relevant information for behavioral guidance.

P3.24

Powerful protective and antioxidant effects of Grapefruit Integropectin on brain cells

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Oxidative stress and neuroinflammation are major mechanisms involved in neurological disorders. In the last years, therapeutic benefits of natural products in brain disorders have been intensively explored. New grapefruit IntegroPectin, derived via hydrodynamic cavitation, characterized by low degree of esterification and enriched in flavonoids and terpenes, largely differ from traditional citrus pectin extracted via acidic hydrolysis in hot water. With the aim of characterizing the beneficial effects of grapefruit IntegroPectin in brain cells, we demonstrated that IntegroPectin rescued cell viability and cell morphology in SH-SY5Y neuronal-like cells treated with H_2O_2. Moreover, IntegroPectin treatment decreased reactive oxygen species (ROS) production and preserved mitochondrial membrane potential severely impacted by H_2O_2 exposure. Similar results were also observed in microglia HMC3 cells. In this cell line, IntegroPectin showed significant anti-apoptotic effects, enhanced cell protection against oxidative stress through the modulation of MAPK/ERK and PI3K/Akt pathways, and exhibited notable anti-inflammatory properties. These results support experimentation of IntegroPectin on preclinical models of complex pathologies marked by extensive phenomena of oxidative stress and inflammation such as neurodegenerative diseases.

P3.25

Ocular and neuronal responses to visual stimuli during free exploration of naturalistic videos in rhesus macaques

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Under natural conditions, eyes are free to explore the visual scene, and both transient pupil dilations and saccadic movements can be influenced by the salience of visual stimuli. Despite its significance, these aspects of visual exploration and the related neural correlates remain poorly investigated. Here we tackle this topic by studying the patterns of ocular behavior of a macaque monkey watching a naturalistic video while recording the neuronal activity from three areas of the frontal cortex (Prefrontal, Premotor Dorsal rostral, and Premotor Dorsal caudal). Different scenes were assigned to four different categories of stimuli depending on the occurrence in the video, which included natural scenes, conspecifics, non-conspecific predators, and non-conspecific harmless animals. Analysis of pupillometry revealed greater dilation when the monkey was exposed to conspecifics compared to both natural landscapes and non-conspecifics, with a further increase in size if non-conspecifics were predator animals. The frequency of saccades followed a similar pattern, since it was higher when predators occurred on the screen with respect to non-predator. The frequency of saccades did not differ significantly between the other categories. Notably, neuronal activity in each brain area was higher for scenes eliciting higher pupil dilatation, suggesting their possible involvement in processing stimulus saliency.

P3.26

Does neuromuscular electrical stimulation influence spinal excitability in Multiple Sclerosis patients with spasticity?

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Spasticity is a common symptom of people with Multiple Sclerosis (pwMS) and is characterised by hyperexcitability of spinal motoneurons. Recent evidence showed that neuromuscular electrical stimulation (NMES) can modulate spinal excitability in healthy people. This study aimed to evaluate the acute responses in spinal excitability, as measured by H-reflex of Soleus (SOL) muscle, in pwMS with spasticity (MS-Sp) and without spasticity (MS), following 3 experimental conditions: isometric contraction (ISO); passive NMES (pNMES); and NMES superimposed onto isometric voluntary contraction (NMES+). For each experimental condition, both MS-Sp (n=15, age=49±9, EDSS=5.2±1.3, MAS=2.3±1.1) and MS (n=15, age=44±12, EDSS=1.3±0.4, MAS=0) performed 15 intermittent plantar flexions at 20% of the maximum voluntary isometric force with the most compromised leg. Before and after each condition, SOL H-reflex amplitudes were recorded. In MS-Sp, H-reflex amplitude significantly decreased after both pNMES (-13%, p=0.007) and NMES+ (-11%, p=0.003), while it was unaltered after ISO (-1%, p=0.498). In MS, H-reflex amplitude did not change following any experimental condition (ISO, p=0.383; pNMES, p=0.323; NMES+, p=0.087). Several inhibitory mechanisms (i.e. presynaptic inhibition, reciprocal inhibition, and recurrent inhibition) may contribute to the reduction of spinal excitability following NMES, suggesting that NMES may be a valuable intervention for improving MS-related symptoms.

P3.27

New insights on the role of AQP4 and its isoforms in cerebral water homeostasis: an *in vitro* and *in vivo* study

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Recent studies highlight the critical roles of AQP4ex and M23 isoforms in AQP4 function. AQP4ex, phosphorylated on Ser335 (p-AQP4ex), is essential for perivascular localization, while M23 is necessary for its aggregation in orthogonal arrays of particles (OAPs). Knockout (KO) mice for these isoforms were used to study their roles in cytotoxic edema at various times. Systemic hyponatremia was induced using acute water intoxication (AWI) and mild water intoxication (MWI) models. Results showed that after 20 minutes of AWI, AQP4ex-KO mice had significantly higher brain water content than wild-type (WT) and AQP4M23-KO mice. In MWI, AQP4M23-KO mice had reduced water accumulation compared to AWI. Western Blot analysis revealed no significant change in total AQP4 expression in WT mice, but AQP4ex increased rapidly after AWI. Interestingly AQP4ex phosphorylation level did not increase and appeared reduced after edema induction. To evaluate whether the increased water content during edema may depend from impaired water removal we injected fluorescent amyloid-β (1-42) into the striatum and measured its diffusion. AQP4ex-KO mice showed significantly slower amyloid-β diffusion and reduced cervical lymph nodes accumulation compared to WT mice. These findings underscore the distinct roles of AQP4 isoforms in brain edema and water homeostasis, with AQP4ex and its phosphorylated isoforms being crucial for astrocyte response to osmotic changes.

P3.28

Initial preclinical development of a novel dual orexin receptor agonist

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Dual orexin receptor agonists effective after systemic administration may be of interest for the treatment of narcolepsy and other diseases and as tools to investigate the physiological mechanisms of orexins in vivo. We designed a novel dual orexin receptor agonist (OX-D, international patent application PCT/EP2023/067633) and performed its initial evaluation. In vitro, OX-D crossed the plasma membrane of murine neuroblastoma cells, suggesting its ability to cross the blood-brain barrier. OX-D elicited intracellular calcium responses in murine neuroblastoma cells transfected with human orexin receptors and its estimated EC_{50} in transfected HEK-293T cells was 68 nM and 11 nM on orexin receptor 1 and 2, respectively. Intracerebroventricular infusion of OX-D (160 µM, 5 µL/h, for 6 h from lights on) in 8 freely-behaving orexin knock-out mice increased wakefulness and decreased non-REM sleep throughout the light period and decreased REM sleep also in the dark period. Subcutaneous bolus injection of OX-D (160 nmol in 1 mL at lights on) in 12 freely-behaving orexin knock-out mice decreased REM sleep during the light and dark periods and decreased the cataplexy-like state in 10 out of 12 mice during the dark period, a finding confirmed in a subset of 4 mice with simultaneous video recordings. These preliminary results suggest that OX-D is a dual orexin receptor agonist with anti-cataplectic activity after subcutaneous administration in orexin-knock-out mice.

P3.29

Postural assessment in a case of bilateral complete amputation of toes

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We studied a 52-year-old professional mountain climber who underwent bilateral amputation of all five toes after severe frostbite. Two tasks were examined: static posturography (SP) and gait initiation (GI), both performed barefoot and with prosthetic shoes. During SP, the participant kept the upright stance for 30 s while an optoelectronic system recorded feet position and body sway, and two force plates measured the Center of Pressure (CoP) displacement of each foot. During GI, the participant stood on the force plates for at least 10 s and then spontaneously started walking; wireless EMG probes recorded the anticipatory postural adjustments (APAs) in trunk and lower limb muscles.
Compared to the shod condition, during barefoot SP, the participant showed a reduced anteroposterior (AP) and mediolateral (ML) extension of the Base of Support (BoS), and the whole-body CoP shifted about 7 mm more anteriorly, approaching the “safer” geometric center of the BoS. Despite this difference, the AP and ML ranges of CoP oscillations were similar in both conditions. In GI, the trunk dorsal muscles showed different APA patterns: when barefoot, they were excitatory in the trailing and inhibitory in the leading side, while bilaterally inhibitory when shod.
Thus, in parallel to the CoP shift toward a “safer” position in SP, the body rotation toward the trailing side in barefoot GI may reveal a more “cautious” approach; this also shows that different postural strategies may be adopted in GI.

P3.30

Synthetic torpor alleviates ischemia-reperfusion injury in Langendorff-perfused hearts

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Torpor, or hibernation, is a reversible hypothermic and hypometabolic state enabling some mammals to survive adverse environmental conditions. Synthetic torpor (ST) can be induced in non-hibernating animals, like rats, by activating excitatory neurons similar to those that induce torpor in mice. Among the physiological adaptations of natural torpor, a cardioprotective mechanism has been described, particularly effective against ischemia-reperfusion injury, a condition characterized by the exacerbation of ischemic damage upon tissue reperfusion.
To explore the cardioprotective effects of ST, 25 Wistar rats were injected in the Preoptic Area of the hypothalamus with either pAAV-CaMKIIa-hM3D(Gq)-mCherry (n=12, ST) or AAV-CMV-GFP (n=13, Control). Three weeks later, Clozapine N-Oxide was administered to induce ST. After 90 minutes, allowing rats to reach the hypometabolic state, hearts were harvested and perfused using a Langendorff apparatus. Global ischemia was maintained for 30 minutes, and after 60 minutes of reperfusion, infarction size was measured using tetrazolium chloride staining. Heart rate was recorded throughout the procedure.
Results indicated that hearts from torpid animals had significantly smaller infarction sizes (21.5 ± 2.9% in ST vs 29.6 ± 2.6% in controls), while heart rate prior to ischemia showed no difference. Thus, there appears to exist a cardioprotective mechanism in ST, at least partially independent of reduced heart workload due to metabolic depression.

P3.31

Schwann cells acquire pro-inflammatory and migrating phenotype, influencing M2 macrophage polarization

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Emerging research suggests that the cellular microenvironment is a complex process influencing cell physiology, with inflammation playing a critical role in cellular growth and neoplastic transformation. With particular emphasis on schwannomatosis (SWN), a rare tumoral disorder characterized by the development of multiple schwannomas of the peripheral nerve, in this study we focused on the physiopathological role of immune cells on Schwann cells (SCs). Herein we explored *in vitro* molecular pathways and biomarkers involved in such inflammatory response, co-culturing monocytes with SCs.
The physiological interaction between Schwann and immune cells is modified at both sides. First, we found that the supernatant harvested from monocytes and M0 macrophages increased the migration and chemotactic response of tumoral SCs and induced the expression of inflammatory genes in SCs. Moreover, when monocytes were co-cultured with SCs, a significant increase in CD163 and IL-10 expression, with significant decrease in IL-12 expression, confirmed the M2 macrophages phenotype of co-cultured immune cells. Overall, we found a significant crosstalk between tumoral SCs and monocytes, demonstrating that schwannoma cells can induce M2 macrophage polarization. Otherwise, SCs develop a pro-inflammatory phenotype and alter their chemotactic migration. Importantly, these evidences could play a crucial role in the development of future therapeutic strategies against neoplasm transformation of the PNS.

P3.32

A central neural network drives arousal from torpor in mice

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Torpor or hibernation is a transient and reversible hypometabolic and hypothermic state, occurring in some species in adverse environmental conditions. Torpor bouts are interspersed with periodic euthermic states (arousal), whose underlying regulatory mechanisms are largely unknown. The aim of this study was to unravel the neural network underlying arousal from daily torpor in mice, by assessing cFos expression in key brain areas known to be involved in thermoregulation and/or autonomic and wake-sleep regulation.
Twelve C57BL/6J female mice (17-24g), were kept at an ambient temperature of 21°C, 12h-12h light-dark cycle (lights on at ZT0) and assigned to one of the following experimental groups: i) Torpor (n=4): the torpid state was induced by fasting (start of fast at ZT12), and mice were euthanized after at least three hours of torpor; ii) Arousal (n=4): mice were induced in torpor and euthanized 90 min. from arousal onset; iii) Control (n=4): mice were fasted, but fasting was unsuccessful in inducing torpor (euthermic). All animals were transcardially perfused and their brains extracted for immunohistochemical detection of cFos. Preliminary results show that in the Arousal group, cFos expression was significantly higher compared to both the Torpor and Control groups in several brain areas involved in thermoregulation and/or autonomic and wake-sleep regulation. These results suggest that arousal from torpor is actively driven by a complex central neural network.

P3.33

The upregulation of GLT-1 expression rescues synaptic plasticity and memory impairment in α7 nicotinic acetylcholine receptors knock out mice

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GLT-1, the main glutamate transporter in the hippocampus, is crucial to maintain synaptic homeostasis during plasticity and memory. Previous studies demonstrated that acetylcholine (ACh) modulates glutamatergic neurotransmission with α7 ACh nicotinic receptors (α7nAChRs) promoting glutamate release. Interestingly, GLT-1 levels are decrease in Alzheimer's disease (AD) models and patients, and α7nAChR dysfunction is linked to AD pathophysiology.
In this study we investigated whether restoring GLT-1 levels might counteract the AD-like phenotype caused by α7nAChR genetic deletion.
We used 12-month-old α7KO mice, and 3xTg AD models and wild type (WT) animals as positive and negative controls, respectively. Mice were chronically treated with ceftriaxone, an antibiotic known to up-regulate GLT-1 expression. We then performed electrophysiological studies of field recordings to assess long-term potentiation (LTP) at CA3-CA1 synapses of hippocampal slices, and behavioral tests to evaluate recognition, spatial and contextual fear memory.
Our findings reveal that ceftriaxone-induced GLT-1 up-regulation reversed the AD-like phenotype in α7KO and 3xTg mice but impaired LTP and memory in WT mice.
These results suggest the presence of a critical dysregulation of glutamate re-uptake at hippocampal synapses in α7KO mice and highlight the need to further explore the interplay between α7nAChRs, Aβ, and glutamatergic transmission in both the healthy and AD brain.

P3.34

Role of Donepezil, a promising antitussive drug candidate, in the central regulation of the cough reflex in the rabbit

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Cough is a purposeful airway defensive mechanism, although without an apparent benefit in cases of chronic cough, impacting the patient’s quality of life. Available antitussive drugs possess low efficacy and severe side effects. Thus, further research is needed to find reliable treatments. Recently, it has been described that acetylcholine (ACh) downregulates cough via muscarinic ACh receptors in the rabbit caudal nucleus tractus solitarii (cNTS). Therefore, it could be interesting to increase ACh levels by inhibiting acetylcholinesterase activity.
In anesthetized, spontaneously breathing rabbits, we investigated the effect of Donepezil, a brain-penetrating, potent and selective acetylcholinesterase inhibitor currently used in Alzheimer's disease treatment, on the modulation of the cough reflex induced by mechanical and chemical stimulation of the tracheobronchial tree. Efferent respiratory activity and electromyographic activity were recorded from phrenic nerves and abdominal muscles. Donepezil, either bilaterally microinjected (5 mM; 30-50 nl) into the cNTS or systemically administered (5 mg/kg; s.c.), transiently increased respiratory frequency and decreased expiratory activity even to complete suppression. More interestingly, Donepezil induced strong depressant effects up to the complete abolition of the reflex.
Our results suggest a possible off-label use of Donepezil in treating cough and provide important hints for developing novel antitussive strategies.

P3.35

Temporal development of functional alterations of midbrain dopaminergic neurons by transgenic expression of neuromelanin in a Parkinson's disease mouse model

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Parkinson's disease (PD) is the second most common neurodegenerative disease. Accumulation of neuromelanin (NM) in dopamine (DA)-releasing neurons of the substantia nigra pars compacta (SNpc) may be the cause of their degeneration. To investigate the temporal development of dysfunctions in the ventral midbrain, we used patch-clamp recordings from horizontal midbrain slices to evaluate passive and active properties of SNpc DA neurons in Tg-TH-hTyr (hTyr) mice expressing the human melanin-producing enzyme tyrosinase in catecholaminergic neurons, at 6-7 and 16-17 months. We identified presumed DA neurons based on electrophysiological hallmarks like a prominent outward current following a strong depolarizing step (I_{AHP}) and a hyperpolarization-activated inward current (I_h). Significant differences were found exclusively in the DA neurons' population of hTyr at both ages, expressed as higher firing rate in response to depolarizing steps. This enhanced neuronal excitability was associated with a significantly smaller I_{AHP} and I_h only in 6-7 months old hTyr mice. In conclusion, our data indicate that NM accumulation leads to higher excitability of the DA neurons at both ages, although at 16-17 months, neurons paradoxically present fewer signs of functional alteration. We propose that at 6-7 months, the population of DA neurons present functionally active neurons, which will later be lost so that at 16-17 months, only neurons somehow more resistant to NM accumulation are recorded.

P3.36

Sleep fragmentation promotes neuronal loss in a mouse model of Down syndrome

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Down syndrome (DS) is characterized by early impairment of brain development, intellectual disability, and a heightened risk of developing Alzheimer's disease (AD) in early adulthood. Sleep is a primary concern for individuals with DS, and poor sleep has the potential to worsen existing DS-related cognitive deficits and to foster development of AD. We investigated in Ts65Dn mouse, model of DS that develops AD-like phenotypes, whether sleep fragmentation (FR) causes neuronal loss in hippocampal regions involved in long-term memory and in areas particularly susceptible to AD-linked neurodegeneration. Adult (3-months-old) Ts65Dn and euploid (Eu) mice were either subjected to FR for three months continuously or maintained in control conditions (CO). At 6 months of age mice sleep and breathing activity were monitored for 24 hours, then dentate gyrus (DG), fields CA3 and CA1, perirhinal cortex (PRC), and lateral entorhinal cortex (LEA) were dissected and analysed for the assessment of cellularity. FR did not alter the wake-sleep structure and the respiratory phenotype in Ts65Dn and Eu mice. Ts65DnFR mice, however, showed a reduction in the number of neurons in the DG, CA1, PRC and LEA compared to control mice, while EuFR mice exhibited neuronal loss only in the DG and CA1. The results suggest that FR may contribute significantly to the worsening of the neurodegeneration typical of AD in DS patients, while it may have a less severe impact in subjects from the general population.

P3.37

Association between muscle oxidative capacity and mortality in older individuals with and without COPD

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The mechanisms by which cardiorespiratory fitness (CRF) contribute to survival are unclear. Impaired mitochondrial function is hypothesized to underlie physical and cognitive decline, and increased mortality risk. Muscle mitochondrial oxidative capacity (k) is reduced with age and in moderate-to-severe COPD, contributing to CRF decline **Aim** To assess the association between k and all-cause mortality in people with and without COPD **Methods** 189 people (87 without COPD; CON). Cox-regression assessed the association of mortality with 6 all-cause mortality predictors: k, FEV_1%pred, activity, 6MWD, BODE index. Confounders: age, sex, BMI, ATS pack-years, O_2 therapy, comorbidity count, status(COPD-CON); and an interaction term between predictor and COPD status. ROC analysis assessed predictor discriminative ability **Results** Interactions were observed for k and FEV_1%pred ($p<0.040$) regression models. k was a significant mortality predictor in CON, while FEV_1%pred in COPD. Across participants, 6MWD and BODE were significant mortality predictors. Time-dependent AUC for k 0.70[0.57-0.82] was similar to other predictors: FEV_1%pred 0.73[0.61-0.85]; BODE 0.74[0.63-0.85]; 6WMD 0.75[0.64-0.85] **Conclusions** We identified a significant all-cause mortality risk reduction with increased k in CON, that was absent in COPD. Survival in CON with below-median k was not different to COPD. The mechanisms associating CRF and survival in COPD may be related to factors other than muscle mitochondrial function.

P3.38

Momast® downregulates AQP3 expression and function in human colon cells

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The water channel AQP3 is an aquaglyceroporin expressed in villus epithelial cells, and it plays a role in water transport across human colonic surface cells. Beyond water, AQP3 can mediate glycerol and H_2O_2 transport. Abnormal expression and function of AQP3 have been found in various diseases often characterized by altered cell growth and proliferation.
Here, the beneficial effects of Momast® have been evaluated. Momast® is a patented natural phenolic complex with antioxidant properties, obtained from olive wastewater (OWW) of the *Coratina* cultivar by applying the principles of a circular economy.
Treatment of human colon HCT8 cells with Momast® at 250, 500, and 1000 µg/ml concentrations reduced cell viability and the tBHP-induced oxidative stress. Confocal studies and Western Blotting analysis demonstrated that treatment with Momast® significantly decreased the staining and the expression of AQP3. Importantly, the AQP3 reduction correlates with a significant decrease in glycerol uptake. Also, preliminary imaging studies using the fluorescent probe HyPer-3, to monitor intracellular H_2O_2 generation, showed that the increase of intracellular fluorescence elicited by external H_2O_2 (50 µM) is partially but significantly reduced in the presence of Momast® or DFP00173, a selective inhibitor of AQP3.
Together, these findings suggest that Momast® might serve as potential adjuvant in colon diseases associated with abnormal cell growth by targeting AQP3.

P3.39

Neuroprotective mechanism(s) of neuroglobin involves the cholesterol metabolism

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Neuroglobin (NGB) is an esa-coordinated heme-protein monomer of 17kDa, belonging to the family of globins. NGB is an inducible protein that exerts neuroprotective effects against several hallmarks of neurodegenerative diseases including mitochondrial functionality and neuroinflammation. So far, no data are available on the putative relation between NGB and cholesterol metabolism in neurodegenerative diseases, another determinant frequently implicated in neurodegenerative syndromes. Previous proteomic data, obtained on breast cancer cells knockout for NGB, identified the involvement of cholesterol metabolism in NGB effects. Here we would uncover whether NGB may directly influence the brain's cholesterol regulatory network. To this aim, SH-SY5Y cells stably overexpressing or not NGB were used as experimental model. Our results indicate that NGB-overexpressing SH-SY5Y cells are characterized by marked modulation in the expression of key proteins regulating cholesterol metabolism. These results support the idea that NGB overexpression promotes increased cholesterol levels in neurons by affecting in concert the mechanisms of biosynthesis and extracellular uptake of cholesterol, raising the hypothesis that cholesterol could be one of the pathways through which NGB exerts its neuroprotective effects.

P3.40

Nutrient availability regulation of osteoblasts function is associated with lipolysis modulation

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Nutrient availability (NA) is critical for osteoblast (OB) mineralization. However, OB metabolic responses to glucose (G) and fatty acids availability remain to be clarified. We assessed the effect of G deprivation (1.25mM) and palmitate (PA,25µM) administration in cultured OB compared to G at physiological concentration (5.5mM). Osteogenesis, OB mitochondrial function, and lipid metabolism were evaluated. We found that LG and LG+PA treatments limited OB proliferation, migration, and clonogenicity compared to G alone. Osteogenesis was enhanced in LG compared to physiological G, while LG+PA showed normal osteogenic function. Inhibition of lipid utilization reduced osteogenesis when G was present at low or physiological levels, highlighting a role for PA utilization. We observed reduced mitochondrial OCR in LG- and LG+PA-treated OB in early differentiation vs G alone, with no differences in ATP production. Increased mitochondrial size in LG vs G conditions was found lately, while LG+PA treatment restored their morphology. Gene expression analysis revealed increased mitochondrial fatty acid and reduced G utilization when G was present in low concentration. This was accompanied by enhanced mitochondrial ROS production in LG and LG+PA compared to G. On the other hand, lipolytic genes were upregulated in LG and LG+PA vs G, with a similar trend in lipogenesis genes, but to a lower extent. In conclusion, OB function is modulated by NA with lipolysis supporting energy demand.

P3.41

Oxidative Stress effects on the hyperglycemia in the brain

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For diabetic patients it is crucial to constantly monitor blood glucose levels to mitigate complications due to hyperglycemia, including neurological and cognitive impairments, leading to psychological stress called "diabetes distress". Diabetes distress can exacerbate the hyperglycemia effects on the brain and negatively impact the quality of life, but the underlying mechanisms remain poorly explored. We simulated diabetes distress in hyperglycaemic zebrafish, along with chronic unpredictable mild stress (CUMS), and evaluated brain redox homeostasis by assessing reactive oxygen species (ROS) content, antioxidant system, mitochondrial biogenesis, and dynamics processes. We also assessed the nuclear factor erythroid 2-related factor 2 (NRF2) content, a critical regulator of redox balance in the brain. Only the combined CUMS+Dextrose challenge, reduced NRF2 levels in the entire brain. Compensatory upregulation of antioxidant genes appeared inadequate to counteract elevated levels of ROS, leading to a lowering of the reduced glutathione content and total antioxidant capacity. CUMS+Dextrose treatment also upregulated transcription factors implicated in mitochondrial biogenesis and dynamics, which is consistent with increased oxidative stress. In conclusion, this study highlights the close interplay between hyperglycemia and psychological distress causing overriding oxidative stress in the brain, rendering the organism vulnerable to the development of disease complications.

P3.42

Fasting metabolic flexibility and fat distribution

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Metabolic flexibility refers to the proper utilization of glucose and fatty acids by mitochondria. It is associated with lean and fit individuals, while patients with obesity and a sedentary lifestyle show a dependence on glucose. We have shown that the respiratory exchange ratio (RER), measured via indirect calorimetry even at rest, can signal differences in the glycemic status and outcomes of patients with prediabetes. Here, we investigate the fat distribution of patients showing lipolytic (RER <0.775) vs. glycolytic (RER >0.925) metabolism at rest.
We studied 15,022 patients, identifying 2,221 patients with lipolytic metabolism and 847 patients with glycolytic metabolism. In normal-weight patients, ultrasound abdominal visceral adipose tissue (VAT) was higher in patients with glycolytic metabolism (+0.41 cm; 95% CI 0.18, 0.63; p<0.001), while subcutaneous adipose tissue (SAT) was lower (-0.16 cm, 95% CI -0.31, 0.01; p=0.32). Also, body fat percentage evaluated through skinfold thicknesses was lower, but not significantly, in patients with glycolytic metabolism (-0.61 %; 95% CI -1.5, -0.26; p=0.2). The same trends were present in overweight and obese patients, although without reaching statistical significance.
Overall, these results reinforce the relationship between metabolic flexibility and metabolic health, possibly showing that the lower fatty acid utilization of patients with glycolytic metabolism can be the result or the cause of higher visceral fat accumulation.

P3.43

3,5-diiodo-L-thyronine (3,5-T2) reverted c-GAS/STING-mediated inflammation in a mouse model of NAFLD

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Consumption of a high-fat diet (HFD) is associated with increased circulating free fatty acids and hepatic lipid accumulation, primarily affecting mitochondrial compartment. Mitochondrial failure not only promotes hepatocellular damage, but also the release of numerous mitochondrial components, such as mtDNA, known as mitochondrial damage-associated molecular patterns or mtDAMPs. They act as ligands for the recently identified cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), which in turn activates the stimulator of interferon genes (STING) to promote inflammation. The thyroid hormone metabolite 3,5-T2 has specific anti-steatotic and anti-inflammatory effects. Here, in a mouse model of NAFLD induced by chronic HFD, we investigated the effect of 3,5-T2 administration on mtDNA damage, which can trigger the cGAS-STING-activated inflammatory cascade, and the related base excision repair system (mtBER). HFD mice showed hepatic steatosis, increased mtDNA damage, exacerbated by mtBER downregulation. Moreover, they exhibited an overexpression of cGAS and STING proteins and of their downstream enzymes, indicating activation of the DAMPs pathway. 3,5-T2 significantly reduced hepatic fat accumulation and restored mtDNA damage through mtBER. Remarkably, 3,5-T2 reverted the activation of inflammatory triggers by reducing cGAS/STING expression. The reported data suggest novel molecular mechanisms underlying the ameliorative effect of 3,5-T2 in inflammatory liver diseases.

P3.44

Impact of yellow prickly pear fruit on glucose metabolism in obese mice

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Functional food can complement conventional treatments to enhance glycaemic control. Accumulating data have shown that chromium picolinate optimizes carbohydrate metabolism, while prickly pear fruit consumption can offer health benefits due to antioxidant and anti-inflammatory properties. The present study aimed to evaluate the efficacy of yellow prickly pear fruit and chromium picolinate, alone or in association, on glucose metabolism in obese mice. Animals were fed a high fat diet (HFD) for 14 weeks and received during the final 4 weeks different oral treatments: water (HFD), lyophilized prickly pear fruit (HFD+PPF), chromium (HFD+CHR), and chromium + prickly pear fruit (HFD+PPFCHR). Another group was fed a standard diet for 14 weeks. We measured body weight, food intake, glucose and insulin levels, glucose tolerance, insulin sensitivity, and insulin receptor protein expression in adipose tissue and liver. The HFD animals showed an alteration of all the analysed parameters. Prickly pear fruit and chromium picolinate significantly improved HFD-induced glucose dysmetabolism by lowering fasting blood glucose and insulin levels, enhancing glucose and insulin tolerance, normalizing the HOMA index and increasing liver and adipose tissue insulin receptor protein expression. No synergistic effect was observed between prickly pear fruit and chromium. These results suggest that prickly pear fruit is a promising natural resource for improving glucose metabolism in obesity.

P3.45

Wide variability in clinical features as a potential pitfall for the diagnosis of rare diseases: the case of two cousins affected by molecularly confirmed Barth syndrome

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Barth syndrome (BTHS) is a rare genetic disease characterized by cardiomyopathy, skeletal muscle weakness, neutropenia, growth retardation and 3-methylglutaconic aciduria. This variable phenotype is caused by pathogenic hemizygous variants of the *TAFAZZIN* gene on the X chromosome, which impair metabolism of the mitochondrial phospholipid cardiolipin (CL). The precise mechanism underlying neutropenia in BTHS is not fully understood, but it is believed to involve deregulation of granulopoiesis. CL deficiency and increased levels of monolysocardiolipin (MLCL) in mitochondrial membranes likely disrupts their function, leading to impaired hematopoiesis and reduced neutrophil production.
Although most BTHS patients are usually diagnosed in the first years of life, the extremely variable clinical picture and the wide range of clinical presentations may both delay diagnosis.
We describe two male maternal cousins sharing the same mutation in the *TAFAZZIN* gene and similar increased levels of MLCL/CL ratio with a wide phenotypic variability. A child early diagnosed with BTHS due to classical early heart failure and an adult with severe neutropenia who received the same diagnosis only at 33 years of age.
Our study corroborates the underestimation of the prevalence of this rare genetic disease (1:1.000.000 males), which should be always be considered in the differential diagnosis of male patients with unexplained neutropenia.

P3.46

Effect of ketosis induced by 1,3-butanediol on brown adipose tissue functionality

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Brown adipose tissue (BAT) plays an important role in controlling metabolism. Factors that influence BAT functionality are only partially known. We examined how ketone bodies affect BAT by inducing ketosis through administering a drinking solution containing 10% 1,3-butane-diol (BD), a precursor of β-hydroxybutyrate, to rats for two weeks. BD-induced ketosis results in a 15% reduction in energy intake, thus we compared the effects of BD to those due to a caloric restriction of the same magnitude (CR). We conducted histological, proteomic (LC-MS/MS), and functional (mitochondrial respiration) analyses. Histology showed that CR increased triglyceride deposition in BAT, while BD reduced it. Proteomic analysis revealed that both CR and BD increased proteins of the extracellular matrix and intermediate filament. CR and BD differed in their influence on metabolic pathways. Compared to control rats, CR enhanced proteins involved in amino acid synthesis, glycolysis, and de novo fatty acid synthesis, while reducing proteins involved in fatty acid and branched-chain amino acid (BCAA) degradation. In contrast, BD treatment increased proteins related to thermogenesis and BCAA degradation and decreased those involved in glycolysis. Mitochondrial respiration in whole BAT homogenate was not significantly affected by CR but was significantly enhanced by BD.
Exogen ketosis profoundly affects BAT physiology, with many metabolic effects being independent of its ability to reduce energy intake.

P3.47

BET proteins as regulators of lysosomal cholesterol homeostasis in Niemann-Pick patients

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Niemann-Pick type C1 disease (NP-C1) is a rare lysosomal disorder caused by mutations in the npc1 gene. This mutation interferes with lipid transport, resulting in the buildup of unesterified cholesterol and other lipids within the endosome-lysosome system. Patients with NP-C1 exhibit visceral and neuropsychiatric symptoms, along with severe cognitive impairment. Currently, there are no curative therapies available. Recent research has revealed that *npc1* mutations produce proteins that are rapidly degraded but still maintain some residual activity. This indicates that increasing the levels of the mutant NPC1 protein could help to restore normal lipid metabolism. We found that an epigenetic pathway regulated by BET proteins (Bromodomains and Extra-Terminal motif) is crucial in controlling the expression of NPC1, which is involved in cholesterol homeostasis. These BET proteins can be targeted with pharmacological inhibitors, such as JQ1, providing a potential therapeutic approach. To investigate its potential, experiments have been conducted using fibroblasts from NP-C1 patients with different NPC1 mutations. The findings showed that inhibiting BETs significantly affected NPC1 expression, cholesterol accumulation, lysosomal size, and NPC1 localization in some patient-derived fibroblasts. This indicates that BET proteins play an essential role in cholesterol metabolism and could be a promising target for treating NP-C1.

P3.48

Disruption of cholesterol metabolism in Parkinson’s disease: a study on neuronal and glial cells

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Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc), leading to dopamine deficiency in the striatum. Besides intracytoplasmic inclusions known as Lewy bodies, oxidative stress, mitochondrial dysfunction and altered protein homeostasis are pathophysiological aspects often observed in the pathology. Although alterations in brain cholesterol metabolism have been associated with many neurodegenerative processes, the specific role of cholesterol in PD remains elusive. Therefore, the aim of this study was to investigate potential abnormalities of cholesterol homeostasis in PD cell models. To this end, SH-SY5Y neuronal, and U373 astrocytic cell lines were used and the Parkinsonian phenotype was reproduced by rotenone administration. The main results show that rotenone treatment significantly increased cholesterol accumulation in SH-SY5Y cells. This event was accompanied by changes in the expression of proteins and enzymes belonging to the cholesterol regulatory network. Conversely, cholesterol content was markedly decreased in U373 cells, suggesting that the PD phenotype may differentially affect the homeostasis of this lipid in neuronal and glial cells. In conclusion, these data demonstrate that cholesterol metabolism is disrupted in PD. Further studies are needed to better understand the potential contribution of such deregulations to PD physiopathology.

P3.49

Kumquat supplementation mitigates high-fat diet-induced neuronal impairment and Alzheimer's disease markers in obese mice

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Although the health benefits of citrus are well documented, few studies examined the biological properties of kumquat, a small citrus fruit, produced by the *Fortunella japonica* tree, that is eaten along with the peel. The present study aimed to evaluate kumquat impact on neuronal damage induced by a high-fat diet (HFD). Mice were fed a normocaloric diet (STD), HFD, or HFD supplemented with 5% kumquat (HFD+K) for 24 weeks. Tunnel assay, pro- and anti-apoptotic gene expression by RT-PCR, insulin signalling protein expression by Western Blotting, and gene expression of Alzheimer's disease markers by RT^2 profiler PCR arrays were evaluated in the differently fed animals' brains. Kumquat supplementation led to a decreased number of apoptotic nuclei in the cerebral cortex, downregulation of Fas-L, Bim, and P27 mRNA and upregulation of BDNF and BCL2 mRNA compared to HFD mice. Additionally, it preserved insulin signalling by increasing the brain protein expression of Ins-R, pAKT, pGSK3B, and pSer-IRS1. PCR-array analysis revealed improved expression of genes involved in β-amyloid generation (Apbb1, Bche, Ide), cell signalling (Apba3, Gnb2, Gnb5, Gng4, Gng5, Gng8, Gng10, Prkcd), and lipid metabolism (Apoe, Igf2, Clu) in HFD+K mice compared to HFD. Overall, kumquat intervention effectively minimized neuronal damage and mitigated risk markers associated with Alzheimer's disease in obese mice.

P3.50

Brain mitochondrial metabolism, neuroinflammation and synaptic plasticity are influenced by the dietary intake of milk in a rat model

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Mitochondria are active players in brain physiology and pathology. In detail, mitochondria located in synapses supply energy to support the synaptic functions, and the plasticity of the nervous system, which is its ability to change synaptic strength and neuronal connections in response to physiological stimuli and environmental changes. Thus the brain, with its lipid-rich content and low levels of antioxidants, is highly susceptible to mitochondrial failure and oxidative stress. Nutritional interventions, which determine a long-term metabolic and inflammatory modulation acting on mitochondrial function, influence brain plasticity and cognitive function. We investigated the impact of the intake of milk, reference food for infant nutrition, on brain mitochondrial functions and redox state. Male Wistar rats were used to explore the effects of isoenergetic supply of milk from cow, donkey or human on mitochondrial function, oxidative stress and inflammation in the brain cortex and the corresponding synaptosomal fraction. Different milk diversly modulates mitochondrial function, efficiency, and ROS production both in brain cortex and synaptosomes. Milk intake modulated the expression of two presynaptic proteins and the levels of main markers of neuroinflammation. These results emphasize the impact of nutrition in brain and synapse physiology, highlighting the key role of mitochondria, nutrient-sensitive organelles able to orchestrate metabolic and inflammatory responses.

P3.51

Cytoprotection by solidified xenon and antioxidant-loaded nanoparticle formulations

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The noble gas xenon (Xe) induces cardioprotection when applied either before or after a myocardial ischemia/reperfusion injury (MIRI) protocol. Due to the scarcity, high cost, and challenging administration of Xe as a gas, we have solidified Xe by incorporating it into cyclodextrin-based nanodevices (CDNs), thus enabling its controlled administration *via* multiple routes.
In this *in vitro* pilot study, we compared the cytoprotective effects of different CDN formulations containing Xe, olive oil, or hydroxytyrosol (HT), a well-known antioxidant. Specifically, we tested their ability to modulate inflammation, oxidative stress, and reduce cell mortality during hypoxia and reoxygenation (H/R) protocols. For this purpose, we utilized H9c2, cardiomyoblasts derived from rat heart, and HMEC-1, human microvascular endothelial cells, exposed to normoxia (5% CO_2 and 21% O_2) or hypoxia (5% CO_2 and 95% N_2) and reoxygenation (H/R), in a hypoxic chamber. Cellular mortality was measured using the MTT assay, and reactive oxygen species (ROS) production was detected using the DCFDA kit assay.
Preliminary results demonstrated a significant reduction in ROS production when cells were pre-treated with Xe, HT, or olive oil, underscoring the comparable cytoprotective and antioxidant properties of these CDN-based formulations. Further studies are warranted to elucidate the mechanisms and protective potential of these compounds in both *in vitro/ex vivo* H/R models and more translational models of MIRI.

P3.52

Mechano-mimetic substrates are a useful tool for early guidance of fibroblast-to-myofibroblast transition

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Fibroblast-to-myofibroblast transition is necessary for physiological wound healing. However, the dysregulation of this process may lead to an aberrant fibrotic transformation. In vivo, fibroblast differentiation is mainly driven by chemical factors, above all, transforming growth factor-β1 (**TGF-β1**); nevertheless, recent studies have highlighted the role of mechanical cues, especially from extracellular matrix (**ECM**).
The aim of this study is to produce a reliable in vitro model for fibroblast differentiation. Conventional glass supports, in fact, do not account for tissue ECM mechanics.
We produced mechano-mimetic substrates of polyacrylamide gels, with different mechanical properties (resulting stiffness: 1÷29 kPa). Subsequently, glass coverslips coated with these hydrogels were functionalized with fibronectin to improve cell adhesion and used to culture **NIH/3T3** fibroblasts in the presence or not of TGF-β1.
Cells were then subjected to morpho-functional analyses; in particular, we focused our attention on the involvement of mechanosensitive ion channels Transient receptor potential canonical 1 (**TRPC1**).
Our result show that fibronectin-coated soft materials (stiffness<1 kPa) triggered a more efficient mechanical cue to induce fibroblast differentiation and induced an increased expression and functionality of TRPC1.
These findings may open the way to high-throughput easily reproducible in vitro models to study mechanisms and strategies to control myofibroblast transition.

P3.53

Chlorogenic acid permeation across intestinal cell monolayers: influence by circadian rhythms in the presence of other natural polyphenols and by dopaminergic neuronal-like cells

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Chlorogenic acid (CGA) is a natural polyphenol potentially health-promoting along the gut-brain axis, limited by poor oral bioavailability. Therapeutics or nutraceuticals polyphenols can influence each other's intestinal absorption following circadian rhythms. We have evaluated, via HPLC-UV analysis, how arbutin, gallic, caffeic and ferulic acids, and circadian entrainment by horse-serum shock, influence CGA permeability across IEC-6 cell monolayers, as intestinal-barrier model. Moreover, neuronal influence on CGA intestinal permeability was investigated mimicking dopaminergic component of enteric nervous system (ENS) by co-culture with PC12 cells. Our results indicate the presence of a circadian-dependent active efflux for CGA intestinal permeation, suggesting its higher bioavailability in the evening rather than in the morning. Among the tested polyphenols, only gallic acid influenced and reduced CGA permeation. These results were consistent with data obtained by orally administration to rats of CGA and gallic acid, where CGA induced an increase of the gallic acid bioavailability, whereas gallic acid decreased the CGA bioavailability both at the bloodstream and central levels. Finally, 60 mM KCl-evoked dopamine release from PC12 cells significantly increased CGA intestinal permeation, downregulating efflux transporters expression/activity, as confirmed by western blot analysis. ENS-released dopamine may enhance CGA oral bioavailability dependence on circadian rhythms.

P3.54

New mechanism of CuZn Superoxide Dismutase (SOD1) secretion via ATP-binding cassette (ABC) A1 transporter in Human T-lymphocytes

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The ability of reactive oxygen species (ROS) to act as cell damaging products as well as intracellular signaling molecules, has been largely demonstrated. In addition, the preferential involvement of peroxides in intracellular signaling pathways has been recognized. Superoxide dismutases represent a large family of antioxidant isoenzymes. Cytosolic CuZn Superoxide Dismutase (SOD1) represents a key intracellular source of hydrogen peroxide (H_2O_2); its involvement in fine tuning of T cell response has been also referred. SOD1 is secreted by many cell lines. The mechanisms underlying its secretion are numerous. In this study we observed that one of this export pathways is mediated by ATP-binding cassette (ABC) family transporters. Our data show that antigen-dependent activation of human T lymphocytes triggers SOD1 production and secretion. Treatment with Glibenclamide, an ABC family inhibitor, on activated human T cells, strongly reduces their SOD1 secretion, by trapping the enzyme in the cytosol. We also observed that this mechanism is preferentially related to the activity of the ABCA1 transporter in T cells. Indeed, in the human neuroblastoma cell line SK-N-BE(2), the treatment with Glibenclamide does not affect SOD1 secretion induced by oxidative stress. These data suggest the involvement of the ABC transporters in SOD1 secretion in activated T cells also proposing the involvement of different secretion routes in neurons in response to oxidative stress.

P3.55

Characterization of an induced Pluripotent Stem Cell line derived from an individual affected by Developmental and Epileptic Encephalopathy carrying the HCN2 p.G460D variant

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Developmental and epileptic encephalopathies (DEEs) are characterized by drug-resistant epilepsy, psychomotor delay, and intellectual deficit, primarily due to pathogenic genes variants in the brain, particularly those encoding ion channels. Several missense variants of HCN channels, crucial for neuronal and cardiac automaticity (I_f current in the heart and I_h current in the brain), have been identified in DEE patients.
This project aims to characterize an induced Pluripotent Stem Cell (iPSC) line with the HCN2 p.G460D *de novo* mutation, causative of the pathology, and demonstrate its pluripotency and differentiation capacity, particularly into beating cardiomyocytes.
The iPSC line was reprogrammed from DEE patient skin fibroblasts using Sendai Viral Vector System (SeV) with Yamanaka factors. Cytogenetic analyses confirmed a normal karyotype, and Sanger sequencing verified the mutation. The stabilized line was free of mycoplasma contamination and SeV. Quantitative RT-PCR and immunofluorescence confirmed pluripotency. An embryoid body formation assay demonstrated the ability to differentiate into the three germ layers. Cardiac differentiation and electrophysiological studies assessed I_f current, and immunofluorescence detected HCN2 channels.
Characterization was successfully completed and confirmed. This cell line can be suitable for future research on excitable cells to elucidate the mechanisms underlying DEE and evaluate the risk of SUDEP development.

P3.56

Melatonin restores KV3.1/KCNC1 channel function in an oxidative stress-related model of cellular aging

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The voltage-gated Kv3.1 channel is expressed in fast-spiking principal neurons and GABAergic inhibitory interneurons throughout the ascending auditory pathway and in various brain regions. Inactivating mutations in the KCNC1 gene lead to forms of epilepsy and a decline in the expression of the Kv3.1 channel is involved in age-related hearing loss. As oxidative stress plays a fundamental role in the pathogenesis of epilepsy and age-related hearing loss, we hypothesized that an oxidative insult might affect the function of this channel. The activity and expression of endogenous and ectopic Kv3.1 were measured in models of oxidative stress-related aging represented by cell lines exposed to 100mM d-galactose. In these models, markers of oxidative stress were dysregulated, while the current density of Kv3.1 was significantly reduced. Importantly, the melatonin reverted all these effects. The reduction of Kv3.1 function was not determined by direct oxidation of amino acid side chains of the protein channel or reduction of transcript or total protein levels but was linked to reduced trafficking to the cell surface associated with Src phosphorylation as well as metabolic and endoplasmic reticulum stress. These data specify Kv3.1 as a novel target of oxidative stress and suggest that Kv3.1 dysfunction might contribute to age-related hearing loss and increased prevalence of epilepsy during aging. The pharmacological use of the antioxidant melatonin can be protective in this setting.

P3.57

Neural conditioned medium and melatonin effects on neural differentiation of human adipose-derived mesenchymal stem cells

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The neural-like differentiation of adipose-derived mesenchymal stem cells (ASCs) has been widely explored in order to develop therapeutic strategies to treat a variety of human pathologies, including nervous system diseases. In this study, a neural-like differentiation was tested by growing human ASCs in conditioned media (CM) from Neural Progenitor Cells (NPC-CM), Olfactory Ensheathing Cells (OEC-CM) or Schwann cells (SC-CM). For each condition, 1 µM melatonin was also added in further samples. Results show that CM treatment was able to increase ASC immunocytochemical expression of typical neural markers such as Nestin, glial fibrillary acidic protein (GFAP), microtubule associated protein 2 (MAP2), and Synapsin I. These increases were differently modulated by the addition of melatonin: a further increase of Nestin, MAP2, and Synapsin I was obtained, whereas an attenuation of GFAP expression was observed. No significant modifications were instead detectable after the melatonin treatment alone. Overall, it can be concluded that a synergistic effect may be exerted by melatonin within an appropriate environment, mainly addressing a neuronal differentiation rather than a glial one. Therefore, this combined strategy provides a further method to develop therapeutical protocols in the field of cell-bases medicine, to be applied for the treatment of neurodegenerative disorders.

P3.58

Ketogenic diet induces an inflammatory reactive astrocytes phenotype reducing glioma growth

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In recent years, the use of the ketogenic diet (KD) has proven beneficial as a complementary approach to standard glioma therapy. Studies have highlighted the interplay between metabolic reprogramming in tumor cells and the surrounding microenvironment, but the mechanisms have not been yet clarified. We investigated the effect of the KD in glioma-bearing mice showing a reduction in tumor growth and an increased median survival rate respect to the mice fed with a matched control diet. To explore a possible mechanism by which KD might exert its function, we proposed glial cells as mediators of the KD effect on tumor growth. Specifically, we describe that KD in vivo, and β-HB in vitro can induce a pro-inflammatory phenotype in astrocytes and that pro-inflammatory astrocytes isolated from the glioma-bearing mice or induced by the β-HB treatment, exhibit increased levels of glutamate transporters which are functionally active in reducing the extracellular glutamate. Moreover, we described increased intracellular basal Ca^{2+} levels in GL261 treated with β-HB or co-cultured with astrocytes, this condition is paralleled by a reduction in tumor microtubes structures among GL261 cells. All these data suggest that β-HB, triggering a pro-inflammatory astrocytes phenotype, can reduce glioma proliferation, excitotoxicity, and glioma connectivity thus proving a beneficial effect on brain parenchyma.

P3.59

Impact of the mutant form of Huntingtin protein on neuronal susceptibility to inflammatory stress

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Accumulating evidence indicates that neuroinflammation acts as both an initiating and a causative factor in multiple neurological diseases. In neurodegenerative conditions, inflammasome activation has been demonstrated in CNS-resident cell types, including microglia, astrocytes, and recently, in neurons. In Huntington's disease models, increased activation of the neuronal NLRP3 inflammasome suggests that neuronal inflammatory pyroptotic cell death potentially contributes to degeneration. Here, we explored the impact of the mutant huntingtin on the inflammatory pathway by using a mouse striatal cell model (STHdh cells) expressing either wild-type HTT (Q7) or the mutant form (mHTT) with 111 glutamine residues (Q111). Results demonstrated that expression of mHTT increase cell susceptibility to inflammatory cell death as demonstrated by the NLRP3 inflammasome activation and pro-inflammatory cytokine (e.g. IL-1β) release. Notably, data also indicate that mHTT leads to parallel mitochondrial structure disruption and increased oxidative stress under both normal and inflammatory conditions. This suggests that the cellular and sub-cellular toxicity of mHTT significantly lowers the threshold for activating inflammatory cell death, consequently increasing the vulnerability of striatal neurons. Overall, obtained findings provide insights into the inflammatory pathway in neurons and suggest that targeting this pathway could have therapeutic potential for Huntington's disease.

P3.60

An omics insight into the expression of the ion transport toolkit in human cerebrovascular endothelial cells

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The Blood-Brain Barrier (BBB) is crucial in neurovascular coupling and for maintaining a controlled environment within the brain InterStitial Fluid (ISF) for reliable neuronal signaling. Key to these processes are intracellular calcium dynamics taking place at the level of brain endothelial cells, which influences BBB integrity by modulating tight junctions and nitric oxide production for vasodilation. However, despite its widely recognized functional role, the molecular actors that enable proper BBB functioning are often overlooked. To identify the main Ion Channels and Transporters (ICTs) contributing to the physiological role of the BBB, we carried out a transcriptional meta-analysis aggregating gene expression data from nine independent and publicly available RNA-Seq studies on the hCMEC/D3 cell line, the most widely used *in vitro* model of BBB. We selected 551 genes coding for ion channels, transporters, membrane ATPases, and some GPCRs. Then, using a tailor-made bioinformatics pipeline, we highlighted a signature expression profile with a potential functional and pathological significance. We identified distinct families of functionally related ICTs underpinning the most distinctive features of the human cerebrovascular endothelium, including the secretion of ISF, the maintenance of BBB integrity, the adaptive regulation of the cerebral blood flow, the uptake and extrusion of trace metals, as well as the membrane components of the endothelial Ca^{2+} toolkit.

P3.61

At the origin of congenital muscular dystrophy: shedding light on the Tdark proteins DPM2 and DPM3

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α-Dystroglycanopathy is a newly emerging subgroup of congenital muscular dystrophies caused by the aberrant glycosylation of α-Dystroglycan (αDG). A defective glycosylation of αDG determines severe muscle damage/degeneration, intellectual disability, epilepsy, and cardiomyopathy. At the origin of glycosylation of αDG there are three proteins, named DPM1, 2 and 3, that form the enzyme dolichol-phosphate mannose (DPM) synthase. Mutations in the genes encoding for DPM2 and DPM3 are indeed associated with defective glycosylation of αDG, and thus with rare congenital muscular dystrophies, together with a complex spectrum of neurological disorders. DPM synthase is a poorly studied enzyme, and even more obscure is the current state of knowledge concerning DPM2 and DPM3 subunits, which are indeed classified as Tdark proteins since their structure, function, interacting molecules and drugs are unknown. To fill these gaps, we set a protocol for transient over-expression of DPM1-3 proteins in HEK293F cells, and for the subsequent purification of the heterotrimeric complex in detergent (LMNG-CHS) micelles. This sample was recently used to obtain preliminary single particle cryo-electron microscopy data that confirmed a homogeneous distribution of particles possessing the expected dimension for DPM synthase in detergent micelles. The 3D structure of DPM synthase will pose the necessary basis for novel therapies targeting this newly emerging subgroup of congenital muscular dystrophies.

P3.62

Glioblastoma mesenchymal subtype tolerates ferroptosis through an increased antioxidant defense

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Glioblastoma (GBM) is an aggressive brain tumor, characterized by heterogeneity and marked resistance to therapy, resulting in poor survival and high mortality. Ferroptosis is an iron-dependent programmed cell death, driven by overproduction of reactive oxygen species (ROS) and lipid peroxidation. Nowadays, an increasing number of studies investigate ferroptosis as a potential novel therapy for cancer, although some tumor types appear to exhibit resistance. Here, we used an *in vitro* approach, to evaluate ferroptosis-inducing effects on two human GBM cell lines, U-251 MG and T98-G, using ferric ammonium citrate (FAC) and erastin. The response to ferroptosis induction was markedly different between the cell lines, indeed T98-G cells, whose profile corresponds to a mesenchymal GBM subtype, showed an enhanced antioxidant defence, with increased glutathione (GSH) levels, as compared to U-251 MG cells, which exhibit proneural characteristics. Moreover, through a bioinformatic approach, we analysed RNA-seq from patient biopsies, demonstrating that mesenchymal GBM showed an upregulation of genes involved in antioxidant mechanisms as compared to proneural one. Our data suggest that GBM subtypes tolerate ferroptosis inducers differently, thus, given the heterogeneity of GBM, it is needed to investigate the molecular aspects of specific subtypes in order to achieve a targeted treatment for each patient.

P3.63

Exposure of human red blood cells to nano- and microplastics: mechanisms of internalization and evaluation of oxidative stress-related effects

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The increased use of plastics in modern times is a serious problem for human health. Red blood cells (RBCs) are the main target for most xenobiotics in the bloodstream. The effects of 1 µg/mL polystyrene nano- (PS-NPs) and micro-plastics (PS-MPs) on human RBCs for 3-24 h were explored. Cellular morphology, binding/internalization of PS-NPs and PS-MPs, oxidative stress parameters, as well as Band 3 protein (AE1/SLC4A1) distribution and anion exchange capability were analyzed. The results obtained showed relevant structural modifications resulting in an increased number of acanthocytes and leptocytes. Both PS-NPs and PS-MPs bound to the RBC plasma membrane, co-localized with estrogen receptors (Erα/ERβ), and were internalized. In addition, an increased trafficking from the cytosol to the RBC plasma membrane, as well as an abnormal distribution of ERs were observed. The exposure to PS-NPs and PS-MPs induced an increase of phosphorylation of ERK1/2 and AKT kinases, consistent with the activation of the ER-modulated non-genomic pathway. The production of ROS, induced by PS-NPs and PS-MPs exposure, caused lipid and protein oxidation, dysfunction of AE1 anion exchange activity, and an increase of methemoglobin levels, thus leading to AE1 clustering. These findings contribute to elucidating potential oxidative stress-related adverse effects of nano- and micro-plastics, which in turn may affect RBCs and systemic homeostasis.

P3.64

Sphingosine-1-phosphate signaling alters the physiological phenotype of circulating monocytes towards an immunosuppressive cluster

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The physiological immune responses can vary according to the biological variability associated with each individual. Based on this concept, the development of specific pulmonary pathologies can rely on the activity of the immune system, impacting the clinical outcome. To understand the variability of the human immune system activity and outlining immunotypes more or less susceptible to immune-mediated diseases, this study was designed starting from bioinformatic analyses of public datasets to evaluate the transcriptomic profile of peripheral blood mononuclear cells (PBMCs) obtained by healthy subjects.
We identified two clusters with different transcriptional repertoire based on an opposite sphingosine-1-phosphate (S1P) metabolism/signaling. Cluster 1 was characterized by an increased S1P metabolism, associated with an enrichment of immunosuppressive monocytes compared to cluster 0. Specifically, only the monocytes ceramidase positive (Cluster 1), key enzyme for S1P synthesis, showed an immunosuppressive phenotype unlike ceramidase negative monocytes (Cluster 0). This study identified a specific S1P-related signature to distinguish different physiological immune profiles that could help healthy individuals to prevent immune-related disorders.

P3.65

Evaluation of early physiopathological effects in mouse spinal cord neurons following proton irradiation

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Radiation neurotoxicity involves early physiopathological effects leading to chronic and latent long-term damage in so-called late response tissues, such as the spinal cord, resulting in myelopathy and severe neurocognitive sequelae. Even with precise therapies like proton therapy, side effects remain a significant area of study. A major concern is that the energy release of protons can be higher at the end of their path, causing off-target effects in healthy tissues. However, the cellular and molecular mechanisms, especially the early physiopathological changes, are still largely unknown. In this study, we analyzed the resident neural population of the spinal cord following incremental doses of proton exposure in two different irradiation settings, representing low and high energy deposition. The results showed changes in the expression of synaptophysin, a major component of presynaptic vesicles in neurons. Synaptophysin, colocalized with the neuronal marker NeuN, was found to be more expressed in the high energy irradiation configuration than in the low energy one. These findings may provide additional insights into early irradiation damage on neuronal plasticity, potentially underlying chronic and late neurocognitive decline.

P3.66

White blood cells AQP3 and AQP9 are involved in NLRP3 inflammasome activation and have relevance as prognostic biomarkers in sepsis

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Sepsis is a multifaceted pathology characterized by severe multi-organ dysfunction caused by the dysregulated host response to infection. Its most severe complication, septic shock, carries an in-hospital mortality rate above 40%. Aquaporins (AQPs) are protein channels that facilitate the transport of water and some solutes across cell membranes. Recent studies using cell and murine models demonstrated their involvement in the NLRP3 inflammasome activation and in the immune cell motility that appear to be impaired during sepsis. Here, we assessed the expression levels of AQP3 and AQP9 in Peripheral Blood Mononuclear Cells (PBMCs) and neutrophils isolated from 48 septic patients. A 30% increase in AQP3 expression ($p < 0.005$) was seen in septic patients compared to healthy donors, while AQP9 showed similar expression levels in both groups. This different regulation of AQP3 was associated with the altered motility and phagocytic activity of macrophages, the primary defense against bacterial infections. On the other hand, the significant correlation between AQP9 and the MEWS2 septic score suggests a potential role in the NLRP3 inflammasome activation and dysregulation during sepsis. Overall, identifying AQPs as novel biomarkers for sepsis could lead to improved prognostic and diagnostic strategies for suspected septic patients. Nevertheless, the emerging role of AQPs in inflammation and the availability of selective blockers suggest their potential as therapeutic targets.

P3.67

Synergistic Anti-Inflammatory Potential of Ketogenic Diet Components on CaCo-2 Cell Line

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Low-carbohydrate diets containing very high percentage of fat and adequate protein intake are called ketogenic diets (KD) due to their ability to stimulate the hepatic production of the ketone bodies acetoacetate (AcAc) and β-hydroxybutyrate (BHB). Ketone body production has various beneficial effects on bowel health, preferring energy sources for colonocytes, maintaining mucosal integrity, promoting satiety, suppressing inflammation and carcinogenesis. The food used in KD contains rich ketone body precursors: coconut oil and Medium Chain Triglycerides (MCT) oil. This study aims to evaluate the anti-inflammatory effects of MCT and BHB and their synergic action on CaCo-2 cell lines, which model intestinal epithelial cells, through cell viability assay, wound healing assay and ELISA test. The CaCo-2 cells were treated with five mM BHB and 0,5 mM MCT in the presence and absence of LPS (1µg/ml) for 24h, 48h, and 72h. Our results showed the anti-inflammatory effects of MCT oil and BHB, increasing cell viability, migration, anti-inflammatory cytokine production, and their synergic effects. We hypothesized that BHB, MCT, and especially their co-treatment had an anti-inflammatory role and trophic action on the CaCo-2 cell line and their synergistic role appears particularly promising, mimicking in vitro the anti-inflammatory action of the KD.

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