

2nd NEUROSCIENCE CONGRESS-2026

CNR – Neuroscience Institute

Padua, 14-16 October 2026

Abstract Book

Talk

October 14th, 2026; 14:20-16:00

Session 1: Neural Circuits: Development, Cognition and Dysfunction

A role for astroglial Ca^{2+} signal dysfunction in familial Alzheimer's disease

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Astrocytes play fundamental roles in brain function and in cerebral tissue homeostasis.

They can sense neuronal activity and release gliotransmitters to modulate neuronal transmission and plasticity, participate in neurovascular coupling to directly link neuronal activity and energy support, and contribute to the metabolic exchanges across the gliovascular unit.

Many functions of astrocytes are strictly coupled to their intracellular Ca^{2+} signaling pathways. For this reason, the study of the possible alterations of Ca^{2+} signal in these cells is of particular interest when investigating the molecular and cellular mechanisms underlying the development and the progression of brain pathologies, such as Alzheimer's disease (AD).

Along this line, we recently characterized in a mouse model of familial AD (B6.152H) a strong reduction of Ca^{2+} signal in cortical astrocytes, associated with deficits in synaptic plasticity and behavior. The disclosure of the underlying molecular mechanism allowed us to develop a LV-based strategy that, by recovering astrocyte physiological signal, was also capable of fully rescuing also the defect in synaptic plasticity. I will here describe novel experimental results based on an improved AAV-based strategy, which allowed us to test whether recovered astrocytes can restore also the defective behavior observed in AD mice.

In addition, I will introduce our new project aimed at characterizing cerebrovascular dysfunction in the same AD model. Vascular defects are frequently observed in both familial and sporadic AD, where they contribute to neuronal degeneration and amyloid accumulation, and ultimately lower the threshold for dementia in patients. As astrocytes are central components of the gliovascular unit, we are exploring the interplay between astrocyte deficits, vascular pathology and AD progression, with a special focus on astrocyte features linked to vascular function, such as the expression and polarization of the water channel aquaporin 4 (AQP4) and the intracellular Ca^{2+} signal at astrocytic endfeet (specialized processes in contact with vessels).

The ultimate goal of our project is to test whether the astrocyte rescue strategy can be beneficial in dampening vascular dysfunction, thus contributing to ameliorate disease progression.

The authors gratefully acknowledge L. Ozmen and F. Hoffmann-La Roche Ltd (Basel, Switzerland) for kindly donating the tg AD mice used in this study.

Systemic immune activation in early adulthood induces long-term synaptic, circuit and neuromodulatory dysfunction in a transgenic mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent form of dementia, characterized by progressive neurodegeneration that leads to cognitive and behavioral impairments. Epidemiological data indicates that females appear to be more vulnerable to AD, with women accounting for approximately two-thirds of patients affected by the disease. Although increasing evidence suggests a link between neuroinflammation and AD neuropathology, the role of systemic inflammation in modulating synaptic plasticity during AD progression remains controversial. Here, we investigated whether an early-life immune challenge influences the development of AD-related alterations in female 3xTg-AD mice. Young adult female 3xTg-AD mice (4 months-old) received a single intraperitoneal injection of polyribinosinic-polyribocytidylic acid (Poly (I:C), 20 mg/kg), a viral mimetic that triggers an innate immune response. Then, Long-term effects were then assessed using biochemical, morphological, electrophysiological, neurochemical, and behavioral approaches. We show that systemic immune challenge with Poly (I:C) induces persistent neuroinflammatory changes, characterized by increased levels of specific cytokines, chemokines, and growth factors in the hippocampus and cortex at 6 and 15 months of age. These alterations were associated with increased phosphorylated tau and reduced dendritic spine density in hippocampal CA1 neurons and frontal cortical pyramidal neurons. Electrophysiological recordings from the medial prefrontal cortex (mPFC) further revealed an increased number of electrically active pyramidal neurons alongside reduced firing frequency and fewer active GABAergic neurons, indicating a remodeling of excitatory/inhibitory balance. In addition, Poly(I:C)-treated mice exhibited decreased kynurenic acid levels and increased extracellular dopamine and serotonin concentrations in the mPFC. Finally, our behavioral data suggest that Poly (I:C) selectively impacts reward-related behavior (i.e., sucrose consumption). Overall, our findings contribute to increasing evidence that neuroinflammation with an altered balance between pro- and anti-inflammatory cytokines contributes to the multifactorial pathophysiology of AD, supporting neuroinflammation as a key component in neurodegenerative disorders.

From timing to wiring: a global molecular code for birth order and neuronal identity in *Drosophila*

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The completion of synaptic-resolution connectomes spanning the entire *Drosophila* central nervous system in both sexes (Berg et al., Cell, 2026; Bates et al., Nature, 2026) has catalogued the morphology and connectivity of essentially every neuron in the fly, revealing more than 11,000 distinct neuron types. This unprecedented resolution sharpens two long-standing questions: how is such extreme cellular diversity generated during development, and what molecular properties endow each cell type with its specific connectivity and function? Answering both requires a complementary atlas, one that pairs each neuron in the connectome with its gene expression profile. We therefore generated a single-cell transcriptional atlas of the fly ventral nerve cord (VNC) and established a high-resolution mapping to these newly completed connectomes. To resolve the granularity described in the connectome, where each cell type contains only 1–2 neurons per VNC side, we profiled over 300k cells across 4 developmental stages, achieving an unprecedented combined 38X coverage. While the atlas should ultimately resolve most cell types, matching them to the connectome at scale will require sustained effort across the research community. Towards this goal, we built a dedicated online platform for browsing, analysing, annotating, and downloading the data to grow this project as an open science initiative. Our analysis of the atlas revealed an unexpected self-emerging feature of the system: neurons within hemilineages form trajectories ordered by birth time. Trajectory analysis led to the discovery that the same 17 transcription factors are expressed in a conserved order across all hemilineages. This points to a strikingly simple rule underlying neuronal diversity: molecular identity emerges from the intersection of a spatial code, i.e. which neuronal stem cell a neuron descends from, and a temporal code, i.e. its birth order within that lineage, marked by a common set of transcription factors. Two coordinates, spatial and temporal, are sufficient to specify a neuron's molecular identity across a nervous system built from thousands of cell types. Initial experiments support an instructive role for this code in specifying neuronal identity across the VNC and central brain. Beyond its instructive function, the code can be used to gain genetic access to neurons and, if proven conserved across species, would offer a new entry point for studying nervous system evolution by identifying "orthologous" neurons as those sharing

developmental (hemilineage) and temporal identity. With synaptic-resolution wiring diagrams of the complete nervous system now in hand, further work on this atlas by the research community will shed light on the molecular and developmental principles governing neural circuit assembly, organization, and function.

Parieto-frontal mechanisms process architecture during dynamic spatial experience in virtual reality

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A central open question in neuroarchitecture is whether specific EEG patterns can decode architectural features experienced during dynamic spatial exploration. Although virtual architectures have been shown to modulate subsequent social cognition (Presti et al., 2023, PNAS), the neurophysiological mechanisms unfolding during the architectural experience itself remain unknown.

Twenty-seven participants experienced a passive virtual promenade through two architectures from a first-person perspective: one progressively expanding and one progressively narrowing. Both conditions elicited comparable visuomotor input while introducing a marked architectural transition between consecutive spatial segments. Two complementary analyses were performed. The first aimed at characterizing local oscillatory power modulations, addressed through time-frequency decomposition with statistical inference via cluster-based permutation testing. The second aimed at characterizing the functional organization of brain networks in terms of functional integration and segregation, addressed through functional connectivity (imaginary part of coherence, iCOH) and network topology metrics (global and local efficiency). Time-frequency analysis revealed gamma-band desynchronization (30–40 Hz) over the left parietal region differentiating the two conditions during both camera speed changes (acceleration, deceleration) and the architectural transition. Global efficiency was systematically higher in the expanding than in the narrowing condition across all frequency bands. Crucially, local efficiency in frontal hubs increased selectively during the architectural transition in the expanding condition, dissociating this phase from the other time windows. While spectral power conflated low-level visuomotor dynamics with the processing of architectural transitions, network-level analysis disentangled them. The selective engagement of parieto-frontal circuits during architectural transitions identifies a network signature of dynamic spatial coding invisible at the level of local oscillatory activity. This finding extends the role of the motor system beyond the coding of immediate action space to the dynamic processing of distal architectural features, providing a neural substrate for how the built environment shapes spatial experience and downstream social cognition.

A flexible system for anticipating a changing environment

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We interact almost flawlessly with an ever-changing environment despite sensory delays, which render incoming information outdated by the time it can be used. The mechanisms behind this feat remain largely unknown. Using complementary techniques, our laboratory is beginning to show that the brain is endowed with specific mechanisms that hold information about upcoming events, regardless of its source or how far in advance it arrives. This mechanism is far more adaptable and resilient than previously thought, and underlies both physical interactions with the world and perceptual judgments. In a related series of experiments, we asked whether information about temporarily occluded moving objects is retained at an early level in the visual cascade. Using an indirect probe, we found that the colour of a disk moving behind an occluder biased the perceived colour of a neighbouring item, suggesting that occluded objects are represented early in visual processing. Together, these findings point to a pervasive system that predicts and bridges gaps in sensory information, and suggest that anticipating the future, rather than reacting to the present, is a fundamental organizing principle of brain function.

October 15th, 2026; 11:00-13:00

Session 2: Molecular and Cellular Mechanisms in Neurological Disease

Repurposing Riluzole and a Novel Sodium Channel Blocker to Enhance Chemosensitivity in Glioblastoma through Modulation of Tumor–Neuron Crosstalk

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Glioblastoma (GBM) is the most aggressive primary brain tumor and remains largely refractory to current therapies because of its high invasiveness and the frequent development of resistance to temozolomide (TMZ). Increasing evidence indicates that GBM cells establish functional interactions with surrounding neurons, promoting tumor progression through electrical activity and glutamatergic signaling. Among the molecular determinants of this tumor–neuron crosstalk, voltage-gated sodium channels (VGSCs) have emerged as promising therapeutic targets.

Here, we investigated the effects of VGSC inhibition in both in vitro and in vivo models of GBM. In primary GBM cells, pharmacological blockade of VGSCs with tetrodotoxin (TTX) induced activation of the ERK signaling pathway, characterized by enhanced nuclear translocation of phosphorylated ERK, increased p53 expression, and marked downregulation of O6-methylguanine-DNA methyltransferase (MGMT), a major determinant of TMZ resistance. Importantly, riluzole (RLZ), an FDA-approved drug currently used for the treatment of amyotrophic lateral sclerosis, reproduced the same molecular effects, supporting its potential repurposing for GBM therapy.

To evaluate the translational relevance of this strategy, we employed an orthotopic murine model of cortical GBM in which electrophysiological recordings were used to assess tumor-induced alterations in cortical activity. Treatment with a newly synthesized VGSC inhibitor (RLZ14) in combination with TMZ significantly improved therapeutic efficacy, reducing tumor progression,

attenuating tumor proliferation, and improving functional outcomes compared with TMZ alone. These effects were associated with modulation of the ERK/p53/MGMT signaling axis, providing a mechanistic basis for the enhanced chemosensitivity. Collectively, our findings identify VGSC inhibition as a promising therapeutic strategy that simultaneously targets intrinsic tumor resistance mechanisms and pathological tumor–neuron communication. The repurposing of riluzole together with the development of the novel analog RLZ14 offers a translational approach to improve the efficacy of standard chemotherapy in glioblastoma.

Losing the column: aberrant lateral connectivity in S1 as a causal and treatable substrate of chronic pain

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Chronic pain (CP) frequently becomes independent of its originating lesion, suggesting that central reorganization, rather than persistent peripheral drive, sustains the pathological state. We tested whether structural remodelling of the primary somatosensory cortex (S1) constitutes a substrate conserved across etiologically distinct CP conditions.

Three models were established in female rats — spinal nerve ligation (SNL), bortezomib-induced peripheral neuropathy (BTZ) and monoiodoacetate knee osteoarthritis (MIA) — spanning neuropathic and nociceptive/inflammatory origins. Chronic 32-channel wireless recordings sampled S1 hindlimb, mPFC, VPL and PAG over eight weeks; mechanical and thermal thresholds were tracked in parallel; cortical microstructure was reconstructed by Golgi-Cox impregnation with circular-statistical analysis of neurite orientation (Rayleigh, Watson-Williams; $N = 200$ neurons/group/region).

All models developed allodynia and hyperalgesia by week 4, accompanied by 2.6–4.4-fold increases in spontaneous firing with model-specific temporal peaks (weeks 2–5). Morphological analysis revealed a consistent reversal of cortical anisotropy: vertical/horizontal neurite ratios shifted from 65%/35% in controls to 42%/58% (SNL), 45%/55% (BTZ) and 52%/48% (MIA), localized to layers II/III and Vb — a somatotopically expansive, intercolumnar architecture. Causal relevance was probed with synchrotron microbeam radiation therapy (MRT; eight 50- μ m microbeams, 120 Gy peak / 2 Gy valley, ANSTO IMBL) delivered to S1 after pain onset. MRT restored 73–96% of normal sensory thresholds in all three models and, in MIA, relieved pain without reversing joint pathology, establishing a cortically specific mechanism. Recovery nonetheless plateaued (~65–70% ceiling), with residual lateral bias and hyperactivity. Aberrant intercolumnar connectivity is thus a conserved, causally relevant — though not exclusive — determinant of chronic pain maintenance.

Exploring the Brain Renin–Angiotensin System: From Molecular Mechanisms to Behavioral Outcomes in Humanized CAG-hACE2 Transgenic Mice

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The brain renin–angiotensin system (RAS) is increasingly recognized as an important regulator of central nervous system functions beyond its classical cardiovascular role. Angiotensin-converting enzyme 2 (ACE2), through the ACE2/Ang-(1–7)/Mas axis, contributes to the balance of RAS signalling and may influence neuroinflammation, cognition and emotional behavior. We investigated the effects of human ACE2 overexpression in CAG-hACE2 transgenic mice, which ubiquitously express hACE2. Male and female CAG-hACE2 mice and wild-type C57BL/6J controls were characterized through biochemical, neuroinflammatory and behavioral analyses. Peripheral cytokines were measured in plasma, while astrocytic and microglial reactivity were assessed by immunofluorescence using S100B and Iba1. Behavioral assessment explored motor, cognitive and emotional domains, including locomotor function, motor coordination, recognition and spatial memory, and anxiety-like behavior. ACE2 overexpression produced a clear sex-dependent profile. Female CAG-hACE2 mice showed significantly increased circulating TNF- α levels, together with enhanced astrocytic and microglial reactivity in the prelimbic cortex, as indicated by increased S100B and Iba1 immunoreactivity, respectively. These molecular changes were associated with an anxiety-like phenotype. In males, astrocytic alterations were also observed, whereas microglial reactivity remained largely unchanged. Male transgenic mice instead showed a selective impairment in short-term recognition memory, while spatial working memory and motor performance were preserved. These findings suggest that sustained ACE2 overexpression does not simply enhance the protective arm of the RAS, but may induce complex, sex-specific adaptations involving peripheral inflammatory signalling, glial responses and neural circuits underlying cognition and emotional behavior. Overall, the CAG-hACE2 mouse represents a useful model to investigate the interplay between brain RAS signalling, neuroinflammation and behavior, and to explore how persistent ACE2 dysregulation may contribute to sex-specific vulnerability or resilience in neuropsychiatric and neurodegenerative conditions.

Differential redox-mitochondrial adaptation to ultra-high-dose-rate irradiation in astrocytes and glioma cells.

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FLASH radiotherapy (FLASH-RT) delivers radiation at ultra-high dose rates (UHDRs) and has been reported to reduce normal tissue toxicity while maintaining tumor control, yet the cellular mechanisms underlying the 'FLASH effect' remain incompletely defined. Here, we compared FLASH- versus conventional radiotherapy (CONV-RT) in murine glioma GL261 cells and primary healthy astrocytic cultures, focusing on redox dynamics, mitochondrial function, bioenergetics, and transcriptional stress programs. Using real-time kinetic assays, flow cytometry, Seahorse analysis, and gene expression profiling, we found that in healthy astrocytes FLASH-RT reduced acute ROS accumulation, limited cell death, and promoted greater recovery of mitochondrial membrane potential and respiratory capacity compared to CONV-RT. FLASH-RT was also associated with sustained induction of cell-cycle checkpoint and senescence-related markers. In GL261 cells, FLASH-RT produced a lower initial ROS peak followed by continued ROS accumulation over time and enhanced G2/M accumulation, together with persistent mitochondrial depolarization and respiratory suppression. Annexin V analysis revealed comparable levels of apoptosis between FLASH- and CONV-RT, consistent with tumor responses being associated with checkpoint-dependent proliferative constraint and metabolic dysfunction rather than enhanced apoptotic execution. Pharmacological inhibition of PARP, calpains, necroptosis, and caspases differentially modulated mitochondrial bioenergetic parameters across modalities and cell types, supporting the contribution of multiple regulated cell death pathways to radiation-induced metabolic stress. Similar stress responses were also observed in human U87 glioblastoma cells, where FLASH-RT induced stronger ROS accumulation, enhanced apoptotic engagement, and alterations in NAD⁺ levels associated with mitochondrial respiratory impairment compared with

CONV-RT. Collectively, these data indicate that FLASH-RT elicits cell-type-specific redox-mitochondrial reprogramming that was associated with relative preservation of mitochondrial function in healthy astrocytes while imposing prolonged metabolic and proliferative constraints in glioma cells, highlighting distinct early redox-mitochondrial adaptations induced by FLASH irradiation in astrocytic and glioma models.

CARescue of Parkinson's disease

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Background: Regulatory T cells (Tregs) play a crucial role in controlling chronic inflammation by suppressing excessive immune responses. In Parkinson's disease (PD), early infiltration of effector T cells into the central nervous system (CNS) exacerbates neuroinflammation and accelerates dopaminergic neuronal loss. This study aims to engineer alpha-synuclein (α Syn)-specific chimeric antigen receptor (CAR) induced Tregs (iTregs) to selectively target neuroinflammation and protect against neurodegeneration in PD models.

Materials and Methods: We generated lentiviral vectors expressing two distinct α Syn-CAR constructs (Nanobody- and scFv-based) combined with Foxp3 expression to transduce primary CD4⁺ T cells isolated from human peripheral blood, driving their conversion into antigen-specific iTregs. α Syn-dependent activation and selective proliferation were confirmed upon specific antigen stimulation, while their functional suppressive capacity was validated through in vitro co-culture assays with effector T cells. Finally, CAR iTregs were intravenously administered into a mouse model of PD induced by a lentivirus overexpressing human SNCA-A53T to evaluate their homing, persistence, and neuroprotective potential in vivo.

Results: Primary human CD4⁺ T cells were successfully transduced, demonstrating high co-expression of CAR constructs and FOXP3. Functional assays revealed that α Syn-CAR iTregs exhibited potent, dose-dependent suppression of effector T cell proliferation under both polyclonal activation and α Syn-specific stimulation. Notably, the Nanobody-based CAR displayed superior antigen specificity and activation kinetics compared to the scFv design. In vivo, systemically injected CAR iTregs selectively migrated to the CNS and accumulated in the substantia nigra. This targeted infiltration led to a significant reduction in microglial activation, lower levels of localized pro-inflammatory mediators, and a reduction in α Syn proteinopathy.

Conclusions: The development of α Syn-specific CAR iTregs represents a novel and promising therapeutic approach to counteract neuroinflammation in PD. By redirecting Treg activity specifically to sites of α Syn pathology, this strategy effectively dampens local immune dysregulation. These findings provide a strong proof-of-concept for the clinical translation of antigen-specific cell therapies in neurodegenerative disorders.

October 15th, 2026; 14:30-16:30

Session 3: Environment, Health and Translational Neuroscience

Rethinking Botulism: The Gut as the First Target of Botulinum Neurotoxins

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Botulism is a severe neuromuscular disease caused by botulinum neurotoxins (BoNTs), traditionally viewed as acting primarily at peripheral neuromuscular junctions after systemic dissemination. Accordingly, the intestine has long been considered a passive entry site. Here, we challenge this paradigm. Using a mouse model of foodborne botulism based on oral administration of BoNT/A and BoNT/B, we show that the intestine is an early and functionally relevant target of toxin activity. Notably, even subclinical doses of BoNTs, insufficient to cause overt paralysis, impair the enteric nervous system, disrupting cholinergic signaling and intestinal physiology, including gut motility, mucus secretion by goblet cells, and intestinal immune defense. Consistent with these findings we also uncover that BoNTs target the gut sensory interface and alter gut-brain communication via vagal afferent pathways. Overall, our data redefine the pathogenesis of foodborne botulism, identifying the gut as the first site of BoNT action and revealing that even low toxin exposure can compromise intestinal defenses.

Prenatal exposure to maternal SARS-CoV-2 infection leads to altered neuro-immune biomarker profiles at birth.

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Maternal infection during pregnancy can induce immune-mediated biological adaptations that influence foetal neurodevelopment. Although vertical transmission of SARS-CoV-2 is uncommon, the impact of maternal infection on foetal neuroimmune signalling remains poorly characterized. We investigated whether prenatal exposure to maternal SARS-CoV-2 infection is associated with alterations in circulating neuroimmune biomarkers detectable at birth. In this case-control study, women with SARS-CoV-2 infection during pregnancy and uninfected controls were enrolled. Maternal and cord blood samples were collected at delivery. Following an initial inflammatory screening, neuro-specific biomarkers (NfL, Tau, P-Tau217, GFAP, S100 β , α -Synuclein) and PTX3 were measured. Correlation network analyses, PTX3 promoter methylation assessment, and haplotype stratification analyses were also performed. Systemic inflammatory mediators did not differ significantly between groups. In contrast, neonates exposed to maternal SARS-CoV-2 infection exhibited significantly altered cord blood concentrations of neuro-specific biomarkers levels compared with controls. Network analyses revealed reduced cytokine connectivity but enhanced interactions among neuro-specific biomarkers, together with stronger maternal-infant biomarker coupling in exposed dyads. Although PTX3 promoter methylation was unchanged, the PTX3 h2/h2 haplotype appeared to modulate biomarker interaction patterns. To our knowledge, this is the first study to characterize a circulating neuro-specific biomarker signature in neonates prenatally exposed to maternal SARS-CoV-2 infection. This supports the utility of neuroimmune biomarker networks as translational tools to investigate the biological consequences of maternal immune activation during pregnancy.

Mechanisms underlying improved glycemic control with combined saxagliptin and dapagliflozin therapy in patients with type 2 diabetes

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Type 2 diabetes (T2D) results from multiple pathophysiological defects, making drugs with complementary mechanisms a rational combination strategy. We investigated why saxagliptin, a DPP-4 inhibitor, and dapagliflozin, an SGLT2 inhibitor, provide greater glycemic benefit when used together in patients with T2D inadequately controlled with metformin. A major strength was the detailed assessment of hormonal and metabolic treatment effects using state-of-the-art techniques for in vivo glucose metabolism and β -cell function. Forty-five participants were randomized to saxagliptin, dapagliflozin, or their combination (n=15 per group) and studied at baseline, after 3 days, and after 4 weeks by a mixed-meal tolerance test with dual glucose tracers. Glucose kinetics were reconstructed by mathematical modeling. A circulatory model estimated glucose clearance and, through a deconvolution-like procedure, endogenous glucose production. Glucose disappearance, urinary glucose excretion and tissue glucose use were derived; after meal ingestion, glucose appearance and metabolic clearance were also computed. Postprandial insulin sensitivity was assessed by PREDIM (an insulin sensitivity index), and β -cell function by modeling insulin secretion. Combination therapy produced a faster and greater improvement in fasting and postprandial glycemia than either drug alone. After only 3 days, postprandial glucose exposure decreased by 36% with saxagliptin plus dapagliflozin, versus 15% and 13% with saxagliptin and dapagliflozin alone. The combination also produced greater improvements in insulin secretion, insulin sensitivity, glucose clearance, postprandial suppression of endogenous glucose production, and intact GLP-1 availability.

The GLP-1 response is of particular interest from a neurophysiological perspective. Previous studies suggest that increased GLP-1 availability during combined SGLT2/DPP-4 inhibition may involve vagal nerve activation. Although not directly tested here, this raises the possibility that part of the metabolic synergy between the two drug classes involves gut–brain neural signaling. Future studies should directly test whether vagal gut–brain signaling contributes to the enhanced GLP-1 response and metabolic synergy of combination therapy. Overall, early combination therapy appears to improve glycemic control by acting simultaneously on multiple complementary mechanisms.

Multidomain Interventions To Improve The Cognitive And Functional Well-Being Of Older Individuals In Long-Term Care Facilities: The I-COUNT Pilot Study

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Enhancing well-being in geriatric care requires integrated, multidisciplinary strategies that simultaneously target physical, cognitive, and nutritional health. The I-COUNT study evaluated the feasibility, acceptability, and preliminary effects of a 6-month multidimensional intervention for older residents in long-term care facilities (LTCFs) compared to standard care. Designed as a cluster randomized controlled trial across two Italian LTCFs, the I-COUNT protocol integrated cognitive and physical intervention, a Mediterranean diet enhanced with functional foods (polyphenol-fortified bread and probiotic artichokes), vaccination promotion, and wearable monitoring technologies. Key evaluation parameters included feasibility metrics alongside clinical outcomes such as cognitive functioning, muscle strength, and physical performance. A total of 90 older adults were enrolled (intervention group: $n=48$; control group: $n=42$). The pilot randomized controlled trial demonstrated a high participant retention rate (86.7%) and strong compliance with physical and cognitive training sessions (median completion $\geq 78\%$). Adherence to the functional food component was more moderate, with a higher consumption rate reported for artichokes (median: 67%) than for bread (median: 50%). While the physical and cognitive programs achieved high acceptability, feedback on the dietary interventions was mixed. At the 6-month follow-up, the intervention cohort exhibited statistically significant differences compared to the control group in the Short Physical Performance Battery (+1.6 vs. +0.3 points, $p=0.001$), handgrip strength (+3.2 vs. -0.1 kg, $p=0.026$), and the Addenbrooke's Cognitive Examination total score (+0.3 vs. -5.5 points, $p=0.040$), though these estimates warrant cautious interpretation given the pilot nature of study and the small sample size. These preliminary findings suggest that our program contributed to improved physical performance and muscle strength (with a potential impact on sarcopenia progression) and had a possible protective effect on cognitive function. Moreover, the I-COUNT study showed that multidomain interventions are safe and well-accepted by LTCFs residents, considering the cognitive and physical domains, while the nutritional protocol requires further refinement. Overall, this pilot work highlights encouraging improvements in physical and cognitive functions, providing valuable baseline data to inform future adequately powered large-scale clinical trials.

Studying lifestyle impact on synapses using proximity labeling-based proteomics

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A healthy lifestyle is the first step towards preserving the physiological homeostasis of the central nervous system. Indeed, moderate physical exercise, coupled with cognitive stimulation and social interaction, preserves learning and memory abilities throughout life, and prevents the onset of a variety of pathologies, including neurodegenerative diseases. The functional, cellular, and molecular mechanisms underlying these benefits can be studied in rodent models by means of environmental enrichment (EE), an experimental paradigm based on providing animals with free access to voluntary physical exercise, along with enhanced sensory stimulation and social interaction.

Reproducing the effect of EE using appropriate drugs holds great translational potential but requires a complete picture of the molecular mediators EE leverages on, and this has not been drawn so far. As a step towards filling this gap, we set up a genetically encoded probe, based on the proximity labeling approach, to isolate the proteome of excitatory synaptic clefts. The miniTurboID biotin ligase was fused to the Lrrtm1 protein to achieve localization at excitatory synapses. This construct was placed under DIO control to trigger expression only upon Cre recombinase-mediated activation. Moreover, the use of tamoxifen-dependent Cre-ERT2 allowed to restrict the time window for expression to the desired interval. After tests in primary neuronal cultures, the constructs were packed in Adeno Associated Viral vectors (AAVs) for in vivo transduction. Expression of TurboID-Lrrtm1 in the hippocampi of mice reared in EE and standard rearing controls enabled retrieval of biotin-labeled synaptic proteins via immunoprecipitation. Samples were subsequently processed for mass spectrometry, followed by comparative bioinformatic analysis. Our datasets of differentially expressed proteins will be further analyzed to determine the key molecular effectors responsible for the positive effect of lifestyle on the healthy and diseases brain, with the final goal of identifying novel druggable targets.

October 16th, 2026; 9:00-11:00

Session 4: From Brain Networks to Organoids: Advanced Models and Technologies in Neuroscience

Network Reorganization After Radiofrequency Thermocoagulation in Regions Supporting Conscious Tactile Perception

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Introduction: Radiofrequency thermocoagulation (RFTC) is a neurosurgical technique used to create focal cortical lesions as a treatment for drug-resistant focal epilepsy [1]. In this study, we leveraged this approach to investigate how targeted cortical disruption influences the neural dynamics underlying conscious tactile perception by investigating the effects induced by RFTC targeted to the parietal operculum and insular cortex—areas known to generate sustained responses to somatosensory input and considered pivotal to the neural correlates of consciousness [2].

Methods: Six patients undergoing stereo-EEG evaluation received electrical stimulation of the median and tibial nerves both before and shortly after RFTC. To assess the impact of the lesion on somatosensory processing, we extracted gamma-band activity (50-150 Hz) evoked by tactile stimulation, focusing on changes in response profiles across both perilesional and remote cortical areas.

Results: Following RFTC, evoked responses were reduced in perilesional regions, reflecting a local functional disruption. In contrast, distant cortical areas showed enhanced activity, especially in the later components of the response (after 50 ms), suggesting compensatory reorganization within the somatosensory network.

Discussion: These findings indicate that RFTC does not solely reduce perilesional processing but also may induce rapid functional redistribution across cortical areas distant from the lesion. Such

network-level plasticity may suggest a resilience mechanism by which the brain preserves essential functions like sensory awareness.

Dissecting the role of mitochondrial calcium signalling in Alzheimer's disease

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Mitochondrial Ca^{2+} signalling is a key regulator of cellular metabolism and intracellular Ca^{2+} homeostasis, yet its contribution to Alzheimer's disease (AD) remains incompletely understood. In particular, how mitochondrial Ca^{2+} handling changes during disease progression and across different brain cell types, and whether these alterations drive or result from broader Ca^{2+} dysregulation, are still poorly defined. Here, we investigated mitochondrial Ca^{2+} signalling in Presenilin 2 (PS2)-based mouse models of familial AD and explored whether modulating mitochondrial Ca^{2+} uptake could restore cellular Ca^{2+} homeostasis. We analysed multiple PS2-based mouse models and disease stages to distinguish alterations associated with PS2 dysfunction from those occurring in the presence of amyloid pathology. Using genetically encoded Ca^{2+} indicators and two-photon imaging in somatosensory cortex slices, we characterized mitochondrial Ca^{2+} dynamics in neurons and astrocytes. We also examined the molecular remodelling of the mitochondrial Ca^{2+} uptake machinery and tested whether overexpression of MICU3, a neuron-enriched regulator of the mitochondrial Ca^{2+} uniporter, could restore neuronal Ca^{2+} homeostasis. Our results reveal a marked stage-dependent remodelling of neuronal Ca^{2+} signalling: mitochondrial Ca^{2+} uptake is reduced at the early, presymptomatic stage and shifts towards enhanced responses as pathology progresses. Neuronal cytosolic Ca^{2+} signals show a similar temporal profile. Preliminary data from astrocytes indicate that mitochondrial Ca^{2+} dysregulation also extends to the glial compartment, suggesting involvement of the broader neuron–glia system. Importantly, MICU3 overexpression in neurons enhanced mitochondrial Ca^{2+} uptake and partially restored cytosolic Ca^{2+} responses at the early disease stage. Together, these findings identify mitochondrial Ca^{2+} dysregulation as an early and dynamic feature of AD pathology that extends across neuronal and astrocytic compartments. Targeting mitochondrial Ca^{2+} handling may therefore represent a strategy to restore cellular Ca^{2+} homeostasis and

counteract functional deterioration during disease progression.

This work is dedicated to the memory of Prof. Tullio Pozzan.

Whole-brain network analysis reveals functional and structural disconnenctions in a mouse model for stroke

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Ischemic stroke represents one of the major causes of mortality and long-term disability worldwide. It arises from a disruption of cerebral blood supply, triggering neuronal and glial injury via multiple cell death pathways.

After stroke, the perilesional tissue undergoes rearrangements and neuronal plasticity mechanisms, playing a crucial role in determining the extent of irreversible injury. Beyond local disruptions, stroke causes both direct and indirect disconnections in functional connectivity, inducing remote dysfunction in structurally intact brain regions that are functionally connected to the site of lesion, and altering whole-brain network communication. However, how these network-level disconnections may contribute to increased functional impairment, thereby modulating post-stroke recovery outcomes, remains poorly described. In this context, our research aims to investigate stroke-induced alterations in neuronal activity at both local and whole-brain network levels.

After characterizing the stroke mouse model of permanent distal middle cerebral artery occlusion (dMCAO), we carried out sensorimotor and cognitive behavioral tasks (gridwalk, water grasping, and Y maze tests), to assess functional deficits induced by the stroke. In parallel, functional neuronal activity patterns were described via widefield mesoscale calcium imaging. We performed a spectral and a functional connectivity analysis, which revealed an altered spectral power and a reduced general functional connectivity after stroke, especially in the subacute phase of recovery. With an integrated approach, we established correlations between the altered neuronal activity and the recovery outcomes, elucidating the relationship between focal lesions and wide-field network alterations. In particular, we characterized the emergence of aberrant activity patterns within the perilesional area and their spread throughout other brain regions.

Generation of patient-specific, iPSC-derived brainstem organoids to study Congenital Central Hypoventilation Syndrome (CCHS)

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Human induced pluripotent stem cells (hiPSC) technology enables in vitro modeling of the human brain through the generation of three-dimensional organoids. This new approach is particularly valuable for investigating those diseases for which in vivo and in vitro models are to date limited or inadequate, such as Congenital Central Hypoventilation Syndrome (CCHS). CCHS is a rare neurodevelopmental disorder of the autonomic nervous system (ANS), characterized by alveolar hypoventilation, due to an impaired autonomic response to hypoxia and hypercapnia, especially during non-REM sleep. The causative gene has been identified in PHOX2B, which encodes for a master regulator of the ANS development and is expressed also in the brainstem centers involved in the autonomic control of breathing.

To generate an appropriate disease model, we first established hiPSC lines from CCHS patients with different PHOX2B mutations and clinical presentations. Then, we differentiated them, alongside control hiPSC lines, into brainstem organoids (hBSO), following a 60 day-long, guided protocol. In this process, cells aggregate into embryoid bodies and undergo neural induction via dual SMAD inhibition, followed by ventralization, caudalization and a final phase of maturation. Our preliminary results from transcriptomic analyses and fluorescent IHC revealed abnormalities in patient-derived hBSOs, including defects in regional patterning and impaired neuronal maturation.

In conclusion, this personalized organoid model provides a powerful tool to investigate CCHS pathogenesis and offers a promising platform for future therapeutic screening and development.

Poster

1. Neurodegenerative Disorders

P1

Clove Bud Essential Oil ameliorates symptoms and improves survival in SOD1-G93A murine ALS models

Maria Lina Massimino 1, *Fiorella Tonello* 1, *Alberto Bernacchi* 2, *Renato Spigarelli* 2, *Maria Chiara Valerii* 2, *Ilaria Saracino* 2, *Anna Kalmykova* 3, *Silvia Turroni* 4, *D'Amico Federica* 4, *Alessandro Bertoli* 3, *Enzo Spisni* 2

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Amyotrophic Lateral Sclerosis (ALS) is a fatal, multifactorial neurodegenerative disease characterized by progressive motor neuron loss, neuroinflammation, oxidative stress, and muscle denervation, ultimately leading to paralysis and death. Since multi-target nutritional strategies may provide crucial supportive approaches to standard therapies, this study evaluated the effects of an early dietary supplementation combining clove essential oil—rich in neuroprotective, blood-brain barrier-permeable compounds like eugenol and beta-caryophyllene—and docosahexaenoic acid (DHA) in hSOD1-G93A murine ALS models.

Treatment started after weaning and continued until the humane endpoint. Motor functionality, physical parameters, and survival were assessed weekly through rotarod, hanging wire tests, and weight measurements, complemented by histological, molecular, and gut microbiota analyses.

The dietary supplement successfully delayed the onset of ALS symptoms, preserved neuromuscular junction (NMJ) integrity, and extended survival by up to 10% of the normal lifespan. Sex-specific analyses revealed that treatment significantly reduced microglial reactivity and altered autophagy and proteostatic gene expression primarily in males, while modulating inflammation-related mRNAs in females. Furthermore, the supplement reduced circulating GDF15, increased beta-NGF (with stronger effects in males), and modulated short-chain fatty acid-producing gut microbiota taxa without any descriptive evidence of hepatotoxicity. Overall, these results demonstrate that a clove essential oil- and DHA-based food supplement can delay ALS progression and improve survival, likely through the synergistic modulation of inflammatory, proteostatic, neurotrophic, and systemic stress-related pathways.

Alzheimer Café: a pilot study in Italy

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Introduction: Community-based psychosocial interventions should be evaluated for their clinical effectiveness, feasibility, and acceptability in routine dementia care. Alzheimer Cafés (ACs) support people with mild cognitive impairment (MCI) or dementia, as well as their informal caregivers, by offering structured social and educational activities.

Aim: To assess the feasibility and acceptability of participating in a multicentre Alzheimer Café programme for one year, as well as its longitudinal effects.

Methods: A 12-month, multicentre, prospective pilot study was conducted in 16 Alzheimer Cafés across eight Italian regions. A total of 168 people with MCI/dementia participated in weekly sessions comprising cognitive, physical, and social activities, while 161 caregivers attended psychoeducational activities and a psychological support group. Longitudinal changes were analysed at baseline and at 6 and 12 months using Generalised Linear Models. Satisfaction with the programme was evaluated at the follow-up stage using a 5-point Likert scale.

Results: More than half of the participants completed the 12-month programme, which supports the feasibility of the intervention. Neuropsychiatric symptoms decreased significantly over time ($p = 0.005$), while quality of life and cognitive performance remained stable. Caregiver's distress declined significantly ($p = 0.001$), whereas caregiver's burden did not change significantly. Satisfaction with the programme was high among both participants and caregivers. The most frequently reported benefits were opportunities for social interaction and emotional support, and improvements in mood and reduced feelings of isolation. Caregivers also valued the availability of meaningful activities for their relatives, as well as the opportunity to share experiences with others facing similar challenges.

Conclusions: The AC model proved to be feasible and highly acceptable in a multicentre, community-based setting. The high level of satisfaction, together with improvements in behavioural symptoms and caregiver distress, supports the role of ACs as a sustainable psychosocial intervention.

Microglia depletion dampens neurodegeneration caused by astroglial selective α Synuclein accumulation by promoting a macrophage neuroprotective state

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Neuroinflammation is increasingly recognized as a key feature of Parkinson's disease (PD), with gliosis and immune activation observed in post-mortem brains. Although neuronal α -synuclein (α Syn) aggregates are well studied, the role of astrocytic α Syn accumulation remains unclear. Astrocytes regulate central nervous system homeostasis and neuroimmune signalling, but their contribution to inflammation during synucleinopathy is poorly defined. We investigated whether astrocytic α Syn accumulation is sufficient to induce dopaminergic neurodegeneration and how astrocytes shape immune responses and peripheral immune cell recruitment. Using an inducible Cre/loxP viral system, we selectively expressed human A53T α Syn in astrocytes. Dopaminergic neuron survival, glial activation, and immune infiltration in the nigrostriatal pathway were assessed by histology, immunofluorescence, and flow cytometry. To test whether astrocytes can independently coordinate immune responses, microglia were chronically depleted with the CSF1R inhibitor PLX3397. Single-cell RNA sequencing of brain CD45⁺ cells was also used to characterize infiltrating immune populations. Astrocytic α Syn overexpression in the striatum caused significant dopaminergic neuron loss in the substantia nigra pars compacta, robust neuroinflammation, and extensive peripheral immune infiltration. Astrocytes acquired a pro-inflammatory transcriptional profile, with increased chemokine expression and activation of immune-related pathways. Microglial depletion reshaped the inflammatory environment and promoted a distinct infiltrating macrophage population (PLX-M Φ) with enhanced phagocytic and immunomodulatory properties. These macrophages accumulated near α Syn aggregates, supported their clearance, and induced an exhaustion phenotype in infiltrating CD8⁺ T cells, reducing neurotoxicity and partially rescuing dopaminergic neuron loss. These findings identify astrocytes as central orchestrators of immune responses in PD and demonstrate that astrocytic α Syn accumulation is sufficient to trigger neuroinflammation and dopaminergic neurodegeneration. Collectively, our results provide a rationale for therapeutic strategies aimed at modulating astrocyte activation and enhancing protective macrophage responses to slow disease progression and preserve dopaminergic neurons.

Targeting peripheral neurodegeneration in Friedreich's Ataxia: a novel mechanism and therapeutic strategy

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Introduction: Friedreich's ataxia (FA) is an autosomal recessive neurodegenerative disorder caused by frataxin (FXN) deficiency, leading to progressive sensorimotor dysfunction. Dorsal root ganglia (DRG) neurons are among the earliest affected cell types, yet the mechanisms underlying their selective vulnerability remain poorly understood. Because FA onset mainly occurs during childhood and adolescence, periods characterized by active neuronal extension, we investigated whether frataxin plays a role in axonal development and cytoskeletal organization. Methods: To investigate frataxin function during neuronal development, we generated an inducible cKO mouse model by crossing Fxn^{flox/flox} with UBC-CreERT2 mice, allowing tamoxifen-induced FXN deletion from P10 to P60. Behavioral, histological, and molecular analyses were combined with studies in human DRG organoids derived from FA patient iPSCs. Pharmacological modulation of microtubule dynamics was also explored to identify potential therapeutic strategies. Results: Frataxin depletion before P25 caused severe ataxia and premature lethality, whereas deletion after P35 was tolerated with minimal motor effects. These findings define a critical developmental window for frataxin function. P15 was therefore selected for mechanistic studies, as its loss at this stage induced severe motor deficits, impaired growth, and degeneration of large DRG neurons, unlike later deletion. DRG neurons from cKO mice and FA iPSC-derived patient DRG organoids exhibited impaired neurite elongation and progressive degeneration. Mechanistically, frataxin loss increased detyrosinated and acetylated α -tubulin levels, indicating microtubule hyperstabilization. To target these alterations, we tested microtubule-modulating drugs and identified a compound that normalized tubulin modifications, improved survival, and reduced neurological deficits. Discussion: Our findings identify a post-developmental window particularly sensitive to frataxin loss, supporting the neurodevelopmental component of FA. This vulnerability likely reflects the role of frataxin in axonal growth and cytoskeletal regulation. Altered tubulin modifications may contribute to impaired neuronal plasticity and neurodegeneration. Importantly, the beneficial effects of a microtubule-targeting compound highlight this pathway as a promising therapeutic target for FA.

Investigating cerebrovascular pathology in a familial Alzheimer's disease mouse model

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Cerebrovascular dysfunction is often associated to Alzheimer's disease (AD) and cerebral amyloid angiopathy is frequently observed in familial AD subjects. Vascular pathology contributes to neuronal degeneration, neuroinflammation and amyloid accumulation, and all these phenomena mutually amplify in AD. Here, we aim to characterize cerebrovascular features in the B6.152H mouse model of familial AD, carrying the APP^{swe} mutation in neurons and the PSEN2 N141I mutation in neurons and astrocytes. Since astrocytes play a fundamental role in the neurovascular unit, we are investigating intracellular Ca²⁺ activity in astrocyte endfeet, specialized processes mediating astrocyte–vascular communication. We previously demonstrated weakened astrocytic Ca²⁺ dynamics in the somatosensory cortex of B6.152H mice and showed that restoration of astrocyte Ca²⁺ signaling is sufficient to fully rescue impaired long-term potentiation in 6-month-old AD mice. The goal of this project is to test whether this rescue strategy can be beneficial also in dampening vascular dysfunction. By combining two-photon Ca²⁺ imaging of astrocytic endfeet, intraluminal labelling with TRITC-dextran and immunohistochemical analysis, we are assessing possible alterations in the cerebrovascular system and in astrocyte signaling at the gliovascular interface. Our analysis reveals local cortical hypoperfusion in B6.152H mice at 8 months of age, despite comparable vessel density. Moreover, preliminary data indicate an altered Aquaporin-4 polarization between endfeet and cerebral parenchyma. We are now investigating astrocyte Ca²⁺ signal at endfeet and developing a protocol to probe neurovascular coupling, to gain fundamental information on functional impairment of the gliovascular unit.

Neurodegeneration in Wolfram Syndrome: Insights from the *Wfs1*^{exon8del} Mouse Model

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Wolfram Syndrome 1 (WFS1) is a rare (prevalence 1 in 160,000–770,000), progressive neurodegenerative disease inherited in an autosomal recessive manner, characterized by juvenile-onset diabetes mellitus, diabetes insipidus, optic nerve atrophy, and hearing loss. WFS1 is caused by mutations in the WFS1 gene, encoding wolframin, an ER membrane protein that protects cells from ER stress-related apoptosis and regulates protein folding quality control. Diagnosis requires early-onset, non-autoimmune insulin-dependent diabetes plus bilateral optic atrophy. Prognosis is poor: most patients die prematurely from severe neurological impairment, including bulbar dysfunction and organic brain syndrome, with median age at death of 30 years, typically from respiratory failure secondary to brainstem atrophy. Neuroimaging and postmortem studies show early medulla, pons, and cerebellum atrophy, linked to central apnea, dysphagia, and impaired smell and taste.

Adults may show psychosis, cognitive impairment, and depression, while younger patients often present sleep disturbances. This heterogeneous symptomatology, combined with the absence of neuro-targeted therapies, underscores the need for biomarkers, therapeutic targets, and outcome measures for future clinical trials. We used the knockout (KO) mouse model *Wfs1*^{exon8del}, which recapitulates diabetic glucose intolerance, anxiety-like behavior under stress, and progressive visual impairment driven by retinal ganglion cell and astrocyte death, to test whether clinically reported brain abnormalities are reproduced in this model, with the final aim of developing a gene therapy approach.

Using stereological quantification, we found reduced parvalbumin-positive neurons in the thalamic reticular nucleus of 12-month-old KO mice—a nucleus regulating thalamocortical circuitry, cortical sleep spindle generation, and γ oscillation modulation. To further probe sleep abnormalities, we are recording locomotor and day/night activity via automated running wheels from 4 months of age, prior to symptom onset.

We are also analyzing brainstem nuclei implicated in autonomic and neurological symptoms—cranial nerve motor neurons (facial, hypoglossal, ambiguous)—and the septal nucleus, which drives hippocampal theta rhythm during REM sleep and aids memory consolidation. This investigation aims to disentangle each region's contribution to WFS1 pathology and identify the most vulnerable brain regions for future therapeutic strategies.

Dysfunction of astrocyte dopaminergic signalling precedes spatial learning deficits in a mouse model of familial Alzheimer's disease

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Spatial learning deficits are an early hallmark of familial Alzheimer's disease (fAD), and are thought to depend on dysfunctional neural networks in hippocampal CA1. Stable spatial representations in hippocampal CA1 neurons rely on astrocyte-mediated regulation of synaptic transmission and plasticity, and may be shaped by dopaminergic inputs conveying novelty and reward signals that facilitate the encoding of salient locations. However, how CA1 neurons, astrocytes, and dopaminergic afferents interact in vivo during spatial learning, and how these interactions are altered during fAD progression, remains largely unknown.

We addressed these questions in the PS2APP mouse model of fAD, comparing age-matched fAD and wild-type controls of 3, 6 and 8 months of age. We first combined cognitive and behavioural testing with in vivo Ca^{2+} imaging in freely moving mice to determine when spatial learning deficits emerge and to identify the neuronal and astrocytic alterations underlying these impairments. We then integrated molecular and pharmacological approaches to examine the contribution of dopaminergic signalling to these changes. Our findings revealed that PS2APP mice develop spatial learning deficits by 8 months of age; these symptoms are preceded by impaired dopaminergic modulation of hippocampal astrocytes, emerging as early as 3 months.

These results identify early astrocytic dopaminergic dysfunction as a potential contributor to cognitive decline in fAD.

miR-30a-5p is enriched in TMEM119-positive plasma EVs from AD patients and promotes neuronal senescence and synaptic dysfunction

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Background. Alzheimer's disease (AD) is characterized by progressive synaptic dysfunction and neuronal loss, yet effective interventions remain limited by the lack of early disease biomarkers and actionable therapeutic targets. Cellular senescence, including microglial senescence, is increasingly recognized as a contributor to AD-related neurodegeneration. Through the release of extracellular vesicles (EVs), senescent or activated microglia may propagate pathological signals, including microRNAs, to neurons. This research aims to investigate the miRNA cargo of circulating TMEM119-positive plasma EVs from AD patients and assess their potential impact on neuronal functions. **Methods and Results.** Plasma EVs enriched for the microglial marker TMEM119 were isolated from individuals with mild cognitive impairment (MCI), AD, and cognitively preserved controls. EV preparations were characterized by nanoparticle tracking analysis, transmission electron microscopy, and immunoblotting. Profiling of 754 miRNAs identified a distinct miRNA signature in TMEM119+ EVs associated with cognitive decline. Among the differentially expressed candidates, miR-30a-5p was selectively enriched in TMEM119+ EVs from AD patients and was functionally investigated in primary hippocampal neurons at different developmental stages. Overexpression of miR-30a-5p during neuronal development (DIV2–5) increased basal intracellular calcium levels and impaired dendritic growth and complexity. At later stages of maturation (DIV7–14), miR-30a-5p reduced synaptic density and presynaptic maturation and induced neuronal senescence, as demonstrated by increased SA- β -gal activity and p16/p21 expression. **Conclusions.** These findings identify TMEM119+ plasma EV-associated miR-30a-5p as a candidate mediator of microglia-to-neuron communication during cognitive decline. Its enrichment in AD-derived EVs and its ability to impair neuronal maturation, synaptic integrity, and cellular homeostasis suggest a potential role in accelerating neuronal dysfunction and brain

aging. TMEM119+ EV-associated miR-30a-5p may therefore represent a promising biomarker candidate and a potential therapeutic target for AD-associated neuronal impairment.

Investigating A β -tau mediated axonal dysfunction in Alzheimer's disease

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Neuronal death in Alzheimer's disease (AD) is linked to the aggregation of amyloid- β and, subsequently, tau hyperphosphorylation and aggregation. In physiological conditions, tau associates with microtubules in axons, regulating their stability and thus representing a key regulator of axonal function. Therefore, we aim to characterize the effect of A β -induced tau hyperphosphorylation on axonal dysfunction by overexpressing the human APP gene carrying a familial AD (fAD) mutation in primary mouse cortical neurons.

Expression of the fAD APP mutant leads to increased tau aggregation in the axons, possibly corresponding to the formation of tau envelopes, as well as α 3-tubulin and neurofilament. Significant defects were also found in the axonal transport of mitochondria, endoplasmic reticulum (ER), LAMP1-positive organelles and synaptic vesicles precursors. Moreover, neurons expressing the fAD APP mutant fail to properly transmit action potentials, although they are over-excitable compared to control.

To further assess the dependency of axonal dysfunction on tau pathology, fAD APP mutant was overexpressed with either a pathological form of tau (P301L), or its phosphodeficient (AP-P301L) or phosphomimetic (E14-P301L) forms. The concomitant overexpression of fAD APP mutant and tau P301L and E14 increases tau aggregation, while tau AP does not.

To explore the reversibility of the observed defects, neurons expressing fAD APP mutant were treated with an inhibitor of p38 MAPK, a kinase involved in tau phosphorylation, which was able to rescue axonal transport and action potential transmission defects.

To assess the dependence of observed defects on increased levels of A β 42, tau aggregation and mitochondria and ER axonal transport were evaluated upon application of A β monomers and oligomers: although all three were affected, they presented some differences with the fAD APP mutant condition suggesting a more complicated picture.

Lastly, the dependence of axonal structural defects on axonal transport alteration was evaluated by overexpressing anterograde motor protein kinesin 1, which was able to rescue the size of tau envelopes, demonstrating that axonal transport defects can induce structural alterations. Further experiments are currently being conducted to characterize axonal dysfunction in vivo: by

using two photon microscopy axonal transport of mitochondria will be investigated in the cortex of 5xFAD mice, untreated and upon treatment with the p38 MAPK inhibitor.

2. Mitochondrial-Nuclear Biology

P10

The Role of UCP4C in Mitochondrial-Nuclear Crosstalk and Metabolic Homeostasis

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Background work. *Drosophila* larvae exhibit constitutive mitochondrial uncoupling. Functionally, UCP4C is essential for development and larva-to-adult progression at low temperatures (15°C). Ucp4C knockdown leads to severe metabolic impairment and developmental arrest under cold stress. Consequently, UCP4C has been proposed as a bona fide uncoupling protein required for thermal adaptation during critical developmental stages.

Current work. Bulk RNA-seq analysis of *Drosophila* larvae across three UCP4C dosages (WT, hemizygous, and knockout) reveals that its loss triggers a dose-dependent transcriptome rewiring centered on nuclear-mitochondrial crosstalk. A trend-based model identifies 2,405 differentially expressed genes (FDR < 0.05), including 271 nuclear-encoded mitochondrial transcripts (~25% of the annotated set). Notably, this subset includes Jig, a mitochondria-to-nucleus shuttling coregulator that controls CrebA nuclear trafficking, linking UCP4C deficiency to disrupted CREB-dependent transcriptional programs. Enrichment for oxidative metabolism pathways (FDR = 2×10^{-21}) positions UCP4C as a node linking mitochondrial uncoupling to ROS homeostasis via the Jig–CrebA axis.

Mitostitch: a new tool to investigate mitochondrial-nuclear communication

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In addition to producing the majority of cellular ATP, mitochondria are key regulators of apoptosis, intracellular signalling and several metabolic processes. It is therefore not surprising that they have evolved mechanisms to coordinate their actions with those of other cellular organelles, including the nucleus. Growing evidence indicates that mitochondria-nucleus communication extends beyond the exchange of soluble signalling molecules, involving specialized mitochondria–nucleus contact sites (MNCs). Recently, molecular evidence for MNCs was proposed, involving the nuclear envelope protein RANBP2 and the mitochondrial VDAC1 channel. These findings implicate the nuclear pore in MNCs and justify further investigations to define their functional role and identify additional molecular components. A major roadblock for these studies is the lack of tools to control mito-nuclear proximity. To overcome this limitation, we developed MitoStitch, a molecular tether that anchors mitochondria to the nuclear envelope using RANBP2 as an anchor, thereby promoting MNCs formation in the vicinity of the nuclear pore, mimicking native contacts. Combining confocal and electron microscopy, FRET-based live cell imaging and biochemical analyses, we validated MitoStitch comprehensively, demonstrating effective perinuclear repositioning of mitochondria, without disrupting their structure or function. This tool provides a novel approach to identify the molecular machinery underlying MNC formation and to explore how mitochondrial subcellular distribution influences the signalling between the two organelles. Engineered cells with inducible MitoStitch expression, paired with transcriptomic and proximity labelling proteomic results, provide powerful means to uncover both the structural components and functional outcomes of mitochondria-nucleus communication. Transcriptomic analyses revealed that perinuclear mitochondrial repositioning reshapes the cellular transcriptional response to oxidative stress, demonstrating that mitochondrial positioning influences the nuclear response to mitochondrial reactive oxygen species (mtROS). Overall, this work establishes MitoStitch as a novel experimental platform for the controlled study of MNC functional outcomes,

providing a powerful tool to dissect the molecular basis and physiological significance of mitonuclear communication.

3. Genetic, Neurodevelopmental and Rare Neurological Disorders

P12

The role of SUMOylation in controlling OPHN1 activity and synaptic function

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Oligophrenin-1 (OPHN1) is a Rho GTPase-activating protein involved in the regulation of actin cytoskeleton dynamics, a process essential for neuronal development and synaptic function. Mutations in the OPHN1 gene cause X-linked intellectual disability (XL-ID), but the molecular mechanisms regulating OPHN1 activity remain poorly understood.

Here, we investigated how OPHN1 activity is controlled under physiological and pathological conditions. Our data indicate that SUMOylation regulates OPHN1 activity by promoting the conformational transition required for its function at excitatory synapses. We further show that the disease-associated G412D mutation, despite not affecting OPHN1 expression levels, alters its structural conformation, impairing SUMOylation and consequently disrupting actin remodeling and synaptic organization. Remarkably, restoring the native conformation of the mutant protein rescues both SUMOylation and OPHN1 function, demonstrating that the structural integrity of OPHN1 is essential for its physiological activity.

Together, these findings identify SUMOylation as a key regulator of OPHN1 and uncover a functional link between protein conformation and post-translational modification in the control of synaptic function. This work provides a mechanistic framework for understanding how OPHN1 mutations lead to synaptic dysfunction in X-linked intellectual disability.

iPSC-derived sympathetic neurons as a model for investigating PHOX2B role in Congenital Central Hypoventilation Syndrome

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A major limitation in studying neurodevelopmental disorder pathogenesis is the lack of models that faithfully recapitulate the complex adult human neurological phenotype and embryonic defects.

Congenital Central Hypoventilation Syndrome (CCHS) is a rare, life-threatening genetic neurodevelopmental disorder characterized by autonomic nervous system (ANS) dysfunctions. 90% of CCHS cases are due to heterozygous polyalanine expansion mutations in the 20 alanine stretch of PHOX2B gene, a master transcription factor of the ANS. Multiple studies suggest that a loss of function mechanism, combined with a dominant-negative effect and/or toxic gain of function of the mutated proteins, is responsible for the entire disease spectrum. No pharmacological treatment is available [1]. PHOX2B-AS1, the natural antisense lncRNA transcribed in the opposite direction and partially complementary to PHOX2B gene, acts by favouring PHOX2B translation, thus a modulation of its expression could represent a new therapeutic strategy aimed at reducing the expression of mutant PHOX2B protein [2]. Given PHOX2B's essential role in autonomic nervous system (ANS) development and respiratory control, PHOX2B knockout animal models are not viable, and conditional models only partially reproduce the CCHS phenotype. Here, we generated autonomic sympathetic neurons (SNs) using induced pluripotent stem cells (iPSCs) derived from CCHS patients carrying different PHOX2B expansions, to investigate possible developmental defects [3,4]. The differentiation process showed morphological abnormalities and alterations in the expression of multiple progenitor and neuronal markers [5]. Furthermore, our preliminary data suggest a defect in migration and proliferation of sympathoadrenal progenitors, as well as in PHOX2B-mediated regulation of different ion channels in the patient lines. The generation of mutant iPSC-SNs also revealed a defective temporal expression of PHOX2B-AS1, along with the appearance of mutant lines-specific

splicing variants in the mutant lines. Overall, our data shed new light on CCHS pathogenesis, paving the way for the identification of possible therapeutic targets.

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- [2] Di Lascio et al. (2026) *FEBS J* 293(12), 3502-3527
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Novel gene therapy strategies to restore full-length atm expression in Ataxia-Telangiectasia

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Ataxia-telangiectasia (A-T) is a rare autosomal recessive disorder caused by mutations in the ATM gene, resulting in progressive cerebellar neurodegeneration, immunodeficiency, and increased cancer risk. The development of effective gene therapies has been limited mainly by the large size of the ATM coding sequence and the need to target both hematopoietic and neuronal compartments.

To address these challenges, we are developing complementary gene therapy strategies to restore full-length ATM expression. We implemented an intein-based protein trans-splicing approach in which the ATM gene is divided into separate fragments that are reconstituted into a functional protein following translation, through intein self-excision. Using a dual lentiviral (LV) system, we achieved efficient reconstitution of full-length ATM in HEK cells and human fibroblasts derived from A-T patients. Functional analyses confirmed that the reconstituted protein is active, restoring DNA damage response signaling. This dual split strategy is primarily aimed at correcting the immunological phenotype in Atm knockout mice via ex vivo gene therapy and autologous transplantation of genetically corrected hematopoietic stem cells. In addition, it may be adapted to target degenerating cerebellar Purkinje neurons in Atm/Top2B-D57N double mutant mice using LVs optimized for retrograde transport from the deep cerebellar nuclei.

In parallel, we developed a triple-split strategy compatible with AAVs, enabling efficient ATM reconstitution in vitro and supporting future translational applications.

To further overcome vector capacity limitations, we are exploring canine adenovirus type 2 (CAV-2) for delivery of the full-length ATM cDNA. Owing to its large packaging capacity and retrograde transport properties, CAV-2 enables efficient targeting of Purkinje neurons following injection into the deep cerebellar nuclei. Collectively, these approaches establish a clinically relevant platform for ATM gene restoration.

Development of a Human iPSC-Derived Neuromuscular Organ-on-Chip Platform for Modeling Friedreich's Ataxia

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Friedreich's ataxia (FA) is a hereditary neurodegenerative disorder characterized by progressive impairment of proprioceptive sensory neurons, motor pathways, and skeletal muscle function, ultimately leading to severe neuromuscular deficits. The lack of physiologically relevant human models that faithfully reproduce the multicellular interactions underlying disease progression has limited mechanistic studies and therapeutic development. Here, we present the development of a human induced pluripotent stem cell (hiPSC)-based neuromuscular organ-on-chip platform designed as a foundation for modeling FA in vitro. The platform combines hiPSC-derived sensory neurons, spinal motor neurons, and skeletal muscle cells within a compartmentalized microfluidic device that enables the reconstruction of both proprioceptive sensory circuits and neuromuscular junctions. Differentiation protocols were optimized to generate robust and reproducible cell populations compatible with long-term culture, while cryopreservation strategies ensured standardized production of neuronal and myogenic progenitors. Microenvironmental parameters, including extracellular matrix composition, cell density, and compartment architecture, were systematically optimized to promote directed axonal growth, formation of stable three-dimensional muscle bundles, and long-term maintenance of neuromuscular co-cultures. The optimized healthy platform will serve as the basis for modeling Friedreich's ataxia using patient-derived hiPSC lines and their corresponding isogenic controls, enabling the investigation of disease-specific defects in sensory and motor connectivity, muscle organization, mitochondrial function, and neuromuscular communication. Functional electrophysiological characterization through microelectrode array (MEA)-integrated chips will be performed to assess network activity and circuit dysfunction. This patient-specific organ-on-chip model provides a scalable and physiologically relevant platform for studying FA pathogenesis and for evaluating novel therapeutic strategies in a human-relevant in vitro system.

Assessing the consequences of AMPARs defective transport in an AP4 deficiency mouse model

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AP-4 deficiency syndrome is a progressive neurodevelopmental disorder caused by mutations in genes encoding the four subunits of AP-4, which regulates cargo sorting along endocytic and secretory pathways. AP-4 indirectly interacts with AMPA receptors, whose trafficking defects are associated with cognitive impairment. We investigated how AP-4 loss affects AMPARs, synaptic transmission, and cognition.

Data showed reduced surface expression of GluA2 both in hippocampal AP4E1 KO neurons and using BS3 crosslinking on acute hippocampal slices from AP4E1 KO mice. Total and intracellular AMPAR levels also tended to be reduced.

Patch-clamp recordings from CA1 pyramidal neurons revealed increased mEPSC frequency and amplitude, with reduced decay time in male KO mice, suggesting altered AMPAR composition, particularly reduced GluA2. Multielectrode array recordings showed increased bursting activity, indicating a shift toward excitation.

Proteomic and biochemical analyses identified autophagy defects and synaptic remodelling. Hippocampi from KO mice displayed increased SNAP25 and reduced PSD95 levels, together with changes in VGAT and VGLUT1, significantly less and more abundant respectively, supporting an excitation/inhibition imbalance.

Behavioural tests revealed anxiety-like and autism-related traits. KO mice spent less time in the center of the open field, showed increased grooming, and exhibited reduced social interaction and discrimination.

To investigate AMPAR fate, we used the ORANGE knock-in toolbox to fluorescently tag endogenous GluA2 and track its trafficking. Reduced colocalization with the endoplasmic reticulum in KO neurons suggests impaired synthesis or organelle-specific degradation.

Overall, our findings identify AP-4 as a key regulator of AMPAR trafficking and stability.

A mechanogenetic strategy to restore synaptic function in PCDH19-Related Epilepsy

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Brain disorders, including epilepsy, are often characterized by altered synaptic connectivity and neuronal network dysfunction. Conventional neuromodulation approaches are invasive and lack cellular specificity, whereas mechanogenetics offers a non-invasive and targeted alternative by exploiting magnetic nanoparticles (MNPs) to mechanically activate engineered synaptic mechanosensors. PCDH19-related epilepsy represents a relevant model for this approach, as loss of PCDH19 disrupts adhesion-dependent signalling, synaptic organization and network excitability, leading to impaired neurotransmitter release and defective synaptic plasticity. Importantly, restoration of these signalling pathways rescues synaptic and functional deficits, highlighting synaptic adhesion complexes as promising therapeutic targets. Based on this rationale, we engineered a membrane-targeted mechanogenetic platform enabling selective coupling of MNPs and controlled activation of synaptic mechanotransduction pathways. In primary neurons, the construct displayed robust membrane expression, preferential localization at dendritic spines and both excitatory and inhibitory synapses, together with selective recruitment of functionalized MNPs. Importantly, no detectable alterations in neuronal morphology were observed, demonstrating the specificity and biocompatibility of the platform. To evaluate its functional effects, the platform was implemented in organotypic hippocampal slices. Conventional low-intensity magnetic stimulation protocols were first validated by MEA recordings, confirming that 10 Hz stimulation increased spontaneous network activity, whereas 1 Hz reduced neuronal activity, in agreement with published studies. The mechanogenetic platform was then expressed in parvalbumin-positive interneurons, where selective MNP recruitment was confirmed. Targeted 10 Hz magnetic stimulation significantly reduced mean firing rate while increasing the inter-spike interval, consistent with enhanced inhibitory control of hippocampal network activity. Together, these findings provide a proof-of-concept that selective activation of synaptic mechanotransduction can modulate neuronal circuits in a cell type-specific manner. Ongoing studies will combine electrophysiology with synaptic calcium imaging and extend this approach to PCDH19 epilepsy models to determine whether mechanogenetic stimulation can restore physiological network function.

Modeling TCF20 and IL1RAPL1 neurodevelopmental disorders in human neurons: from disease mechanisms to gene restoration strategies

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Mutations in TCF20 and IL1RAPL1 are important genetic causes of neurodevelopmental disorders, including intellectual disability (ID) and autism spectrum disorder (ASD). Although both genes are essential for normal neuronal development and synaptic function, the cellular mechanisms by which disease-associated variants disrupt human brain development remain poorly understood, and therapeutic strategies are still lacking. We aimed to define the molecular and functional consequences of TCF20 and IL1RAPL1 mutations using complementary human cellular models while developing innovative gene restoration approaches.

We employed isogenic human induced pluripotent stem cell (hiPSC)-derived neurons and patient-derived cell lines to investigate how TCF20 deficiency and disease-associated truncating mutations affect neuronal differentiation, dendritic maturation, synaptic organization, and gene expression. In parallel, we characterized a panel of newly identified IL1RAPL1 patient variants to determine their effects on protein stability, subcellular localization, trans-synaptic signaling, dendritic spine development, and synapse formation. Structural and molecular analyses have been combined with functional studies using high-density multielectrode arrays (HD-MEA) and transcriptomic profiling to identify convergent pathways underlying neuronal dysfunction.

Finally, we developed and optimized split-intein dual-AAV strategies for the reconstitution of full-length TCF20 and IL1RAPL1 proteins, overcoming the size limitations of conventional AAV vectors. The therapeutic potential of these approaches will be evaluated by assessing their ability to rescue molecular, structural, and functional deficits in human neuronal models.

Effect of PHOX2B frameshift mutation in the development of autonomic neurons from CCHS-patient derived iPS cells

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PHOX2B is a homeo-domain transcription factor, whose expression is required for the development of neural structures that regulate the cardiovascular, respiratory and digestive organs, forming the sensory and motor arms of the visceral reflex circuits. Mutations (frameshift, missense, nonsense) in the coding sequence of PHOX2B have been identified in several neurocristopathies, due to a defect in the development of the neural crest cells (NC), such as Hirschprung's disease (HSCR) and neuroblastoma (NB), a tumour that represents 10% of all the pediatric tumours. The risk for these diseases dramatically increases in patients with Congenital Central Hypoventilation Syndrome (CCHS, MIM #209880) harbouring a non-polyalanine repeat expansion (NPARM) mutation of PHOX2B, but the molecular mechanism of pathogenesis remains unknown. Conversely, poly-alanine repeat expansion mutations (PARM, ranging from +5 to +13 alanines) are almost exclusively associated with isolated CCHS.

Among NPARMs, frameshift mutations (FS) form a group of heterogeneous gene variations; they can be insertions/deletions of one/few nucleotides or longer stretches, and produce two different kinds of aberrant translational frames, resulting in an elongated or a truncated protein, whose additional or reduced length depends on the model of frame. In vivo studies provided several evidence of the functional differences between PARM and NPARM mutations, and this can be explained, at least in part, by differences in the transcriptional properties of the PHOX2B mutants. So far, most of the knowledge on CCHS and associated neurocristopathies derives from human cell cultures and murine models of the disease: both these approaches provide a limited representation of human pathophysiology. By using an established and optimized protocol in our laboratory, we generated sympathetic neurons from patient-derived iPSCs bearing the p.Ser311Glufs*49fs PHOX2B mutant variants, which displayed impaired maturation and altered RNA signature compared with controls. We also demonstrated that the mutant PHOX2B protein has an increased molecular weight due to the addition of 44 amino acids and it may form intracellular aggregates that could sequester the wild-type protein. All these data will contribute to the identification of networks that are altered by the presence of NPARM PHOX2B mutation

and for the identification of new molecular biomarkers to use as prognostic factors or as new pharmacological targets.

Molecular and Synaptic Drivers of Angelman Syndrome Pathogenesis

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Angelman syndrome (AS) is a severe neurodevelopmental disorder caused by the loss of maternal UBE3A expression. However, the precise molecular mechanisms by which UBE3A loss disrupts brain development remain poorly understood. Here, integrating mouse and human neuronal models, we dissect the cell-autonomous roles of UBE3A in synapse development and circuit specification. Using single-cell genetic manipulations and in vivo imaging, we demonstrate that the subcellular localization of distinct UBE3A isoforms dictates specific programs of excitatory and inhibitory synapse maturation. Building on these findings, we investigate the early molecular cascades that precede circuit dysfunction and behavioral phenotypes in AS models. Combining biochemical profiling and multi-omics analyses, we demonstrate that neuronal sumoylation, which is a post-translational modification with a key role for brain development and function, is compromised during early postnatal development, directly altering the transcriptional and epigenetic landscapes. Together, our study establishes isoform-specific UBE3A dynamics and sumoylation dysregulation as core drivers of synaptic pathology, offering novel mechanistic insight into AS pathogenesis.

PCDH19 in Parvalbumin-Positive Interneurons Is Required for Cortical Network Function and Social Behavior

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Protocadherin-19 (PCDH19) is a cell adhesion molecule involved in neuronal development, synapse organization and circuit assembly. Pathogenic variants in PCDH19 cause developmental and epileptic encephalopathy 9 (DEE9), a neurodevelopmental disorder characterized by infantile-onset epilepsy, autism spectrum disorder, cognitive impairment and psychiatric manifestations.

Here, using a conditional mouse model with selective deletion of *Pcdh19* in parvalbumin-positive interneurons (PVIs), we demonstrate that PCDH19 is required in these interneurons for normal brain function. Loss of PCDH19 in PVIs is sufficient to induce spontaneous EEG abnormalities, increased seizure susceptibility and autism-like behavioural deficits. In the medial prefrontal cortex (mPFC) PCDH19 deficiency in PVIs remodels excitatory synapses, enhances excitatory drive onto PVIs and disrupts the developmental maturation of network activity. In vivo calcium imaging further reveals abnormal recruitment of PVIs during social interaction. Finally, chemogenetic enhancement of PVI activity during a defined postnatal developmental window restores normal social behaviour despite the persistence of the underlying genetic defect.

Together, these findings establish a critical role for PCDH19 in PVI function and prefrontal circuit maturation, link PVI dysfunction to impaired social behaviour, and show that enhancing PVI activity during postnatal development is sufficient to recover normal sociability.

Cerebellar Signatures of Sensory Dysfunction in Autism

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Sensory abnormalities are highly prevalent and disabling in autism spectrum disorder (ASD), profoundly disrupting learning, social engagement, and environmental adaptation. Although mechanistic research has largely focused on primary sensory cortices, perception fundamentally relies on coordinated dynamics across distributed neural networks. The cerebellum is central to this process, regulating sensory timing, prediction, and the integration of expected versus incoming signals.

Here, using awake wide-field calcium imaging in a Shank3-haploinsufficient mouse model of ASD, we show that impaired whisker-dependent discrimination is associated with reduced cortical responsiveness and defective temporal adaptation in the primary barrel field cortex (SSp-bfd). Concurrently, sensory stimulation triggered an anomalously delayed response in the cerebellum. Pharmacologically inactivating SSp-bfd completely abolished this late cerebellar signal, pointing to pathologically reverberating activity across cerebro-cerebellar networks. Resting-state functional imaging further substantiated this circuit-level dysfunction, revealing hyperconnectivity within both cortical and cerebellar regions.

Together, these findings demonstrate that Shank3 deficiency disrupts large-scale spatiotemporal communication across cerebro-cerebellar networks rather than acting through isolated regional deficits. Ultimately, our work establishes a mechanistic foundation for developing quantitative, network-level physiological endpoints to support patient stratification and evaluate targeted therapies.

Maternal Immune Activation Affects Mesocorticolimbic Circuit Functionality in Anesthetized and Awake Mice

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Maternal immune activation (MIA) caused by prenatal infections may alter fetal physiological development by interfering with cytokine cascade balances, increasing the risk of neurodevelopmental disorders (NDDs) in offspring. MIA raises the risk of impaired neuronal function, involving the mesocorticolimbic system among other vulnerable neural trajectories. Within this circuit, projections relating the ventral tegmental area (VTA) to the medial prefrontal cortex (mPFC) are essential for cognitive, social and reward processes. Disturbances to these neural substrates could result in abnormal behaviors downstream. As multiple NDDs exhibit similar features, shared pathogenic mechanisms might involve a dysregulation of this very system. Preclinical evidence suggests that MIA could alter mesocorticolimbic functionality and predispose to an NDDs-like phenotype. Therefore, our aim is to verify this possibility in a MIA model induced by gestational exposure to poly (I:C). We examined the impact of MIA on the spontaneous electrical activity of putative (i) VTA dopamine and (ii) mPFC pyramidal neurons, alongside on the (iii) mPFC population dynamics. Pregnant C57BL6/J mice were administered i.p. poly (I:C) on gestational day 12, while control dams received vehicle solution. We performed in vivo single-unit extracellular recordings of VTA dopamine and mPFC pyramidal neurons in anesthetized offspring. Bulk calcium transients from mPFC population were recorded via fiber photometry in awake head-fixed GCaMP-expressing mice, receiving sucrose and quinine as rewarding and aversive stimuli. Prenatal MIA disrupts VTA neurons' electrical activity in both their tonic and bursting modes. However, the electrical phenotype of mPFC cells appears unaffected. These data are integrated with cortical population recordings, where signal amplitude and duration of mPFC calcium activity are driven exclusively by cue valence. Yet prenatally challenged mice show an increased discrimination index between stimuli, indicating a maladaptive neural process underlying these responses. Given that alterations in dopaminergic neurotransmission could lead to abnormal inputs to mPFC, this may impair circuit fine-tuning efficiency rather than cause gross deficits. Hence, our results demonstrate MIA impact on mesocorticolimbic functionality. However, a broader characterization of the induced phenotype is required to clarify MIA role in the pathophysiological features shared by several NDDs.

Base-editing mediated MECP2 gene correction as a novel gene therapy strategy for Rett syndrome

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Gene therapy represents a promising approach for Rett syndrome (RTT), as MeCP2 restoration ameliorates disease phenotypes in preclinical models — yet regulating MECP2 expression remains challenging due to its cell type-specific patterns and tight dosage requirements in the brain.

To address this, we are developing an adenine base editing (ABE) strategy to restore endogenous MECP2 expression. RTT-causing mutations are notably biased toward ABE-addressable variants: the eight most frequent hotspots are C-to-T transitions, and together with G-to-A variants, ABE could potentially correct over 70% of pathogenic mutations.

As proof of concept, we targeted five common MECP2 mutations (T158M, R168X, R270X, R306C, R133C). sgRNA screening in engineered cell lines achieved 30-80% editing efficiency, but bystander editing at the T158M and R133C loci emerged as a major limitation, negatively affecting the efficiency of MeCP2 protein restoration.

To overcome this, we screened a library of TadA-mutant ABE variants to identify engineered editors with enhanced single-nucleotide specificity and reduced bystander activity, tailored to each target context. The most promising variants significantly improved precision in engineered cell lines, efficiently avoiding bystander A>G changes. Since the T158M locus is conserved between human and mouse, we further evaluated these editors in primary neurons from Mecp2^{T158M/y} mice, reducing bystander editing from 60% to around 10%, with corresponding improvements in Mecp2 protein restoration. We are now translating these advances in vivo via a dual-AAV split-intein delivery system, achieving up to 28% editing efficiency in the cerebral cortex after neonatal ICV injection. To date, Mecp2 appears to be partially restored at heterochromatic foci in the cerebral cortex and hippocampus of treated mice. Moreover, restored Mecp2 at the cerebral cortex level shows increased DNA binding affinity compared to untreated mutant control mice.

Ongoing work includes validation in human iPSC-derived neurons and further editing window restriction optimization in these cells. Together, these findings highlight ABE as a promising

therapeutic strategy for RTT, while underscoring the need for improved precision to ensure reliable therapeutic efficacy.

4. Motor, Sensory and Mirror Networks

P25

Behavioral Relevance Coding in Human Area 46 Precedes Selective Motor Activation to Action Targets

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The lateral prefrontal cortex (LPFC) actively contributes to the adaptive control of goal-directed behavior. Evidence suggests that the LPFC encodes the behavioral relevance of stimuli, distinguishing action targets from irrelevant objects; however, how this selectivity emerges over time and integrates within large-scale cortical dynamics underlying action preparation remains unclear. We recorded stereo-electroencephalography (sEEG) activity from 43 patients affected by drug-resistant epilepsy while they prepared to grasp an object and compared it with passive observation of the same object. Gamma-band responses were used to characterize cortical responsiveness and to track the spatiotemporal propagation of activity during action preparation. Neural activity first emerged in the occipitotemporal cortex in both experimental conditions and then progressed along two parallel pathways: an intraparietal and a prefrontal one. The intraparietal pathway showed highly similar dynamics during both action preparation and passive observation, suggesting largely intention-independent visuospatial processing. In contrast, the prefrontal pathway exhibited progressively stronger selectivity for behaviorally relevant objects as activity advanced rostrally. Within this pathway, area 46 exhibited sustained responses selectively associated with action-relevant objects, preceding the similarly selective engagement of premotor and motor regions. Overall, our findings identify area 46 as a key node in whole-brain dynamics that orchestrates action preparation by integrating object relevance into executive control signals guiding premotor and motor engagement.

Functional Near-Infrared Spectroscopy Reveals Stable Visually-Evoked Cortical Responses across Circadian Phases

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Circadian rhythms exert a pervasive influence on human behavior, physiology and cognition. Multiple cognitive and sensory domains, including alertness, attention, reaction time, working memory, executive function, and visual processing (e.g., contrast sensitivity and temporal resolution), show robust circadian modulation. Performance is typically enhanced during an individual's circadian optimal peak and impaired during circadian troughs, likely due to interactions among circadian regulation of arousal, neuromodulatory tone, and homeostatic sleep pressure (Wright et al., 2002). At the neural level, electrophysiological studies have documented circadian influences on cortical excitability and oscillatory activity, but their influence on sensory-evoked neural activity remains poorly understood. In fact, it remains unsolved whether these behavioral and neural fluctuations reflect genuine modulation of early sensory processing or instead arise from downstream cognitive, attentional, or motivational mechanisms. In this study, we investigated whether visually evoked cortical responses measured with functional Near-Infrared Spectroscopy (fNIRS) are modulated by circadian phase or altered by repeated measurements across time. Hemodynamic responses over the occipital cortex were recorded in healthy adults during a robust flickering visual stimulation paradigm. Participants were assessed either (i) at three circadian timepoints within a single day and again across multiple days at matched hours, or (ii) across short-, medium-, and long-term test–retest intervals, spanning five minutes, two months, and two years. Across all analyses, visual stimulation reliably elicited the canonical hemodynamic response, characterized by increased oxygenated and total hemoglobin and decreased deoxygenated hemoglobin. Critically, response amplitudes showed no systematic variation across circadian phases or retest intervals, and correlation analyses revealed strong consistency across sessions. These findings demonstrate that early visual cortical responses measured with fNIRS are remarkably stable over time and resilient to circadian influences and task repetition. The pronounced temporal reliability of fNIRS-based visual responses supports their use in longitudinal, multi-session, and time-of-day-variable studies, and highlights fNIRS as a robust, non-invasive biomarker of sensory cortical function.

Experience-dependent changes in CA1 neuronal activity during visual perceptual learning

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A central question in neuroscience is how sensory information is transformed into neural representations that support perceptual decisions and adaptive behavior. Although the hippocampus is classically associated with episodic memory, its contribution to sensory perceptual learning remains unclear. Here, we investigated hippocampal involvement in visual perceptual learning using a two-alternative forced-choice task in mice.

Animals were first trained to discriminate a rewarded vertical grating from an unrewarded horizontal grating. After reaching criterion performance, they were tested with progressively more difficult discriminations in which the unrewarded stimulus had the same spatial frequency as the rewarded stimulus but increasingly similar orientations. Behavioral performance was high for clearly distinct stimuli and progressively declined as orientation differences decreased, allowing estimation of each animal's perceptual discrimination threshold. To examine the hippocampal neuronal correlates of perceptual learning, we performed in vivo single-photon calcium imaging in the CA1 subregion while mice carried out the task. Neuronal activity was compared between an early, non-expert stage, when animals were naive to ambiguous stimuli, and a later, expert stage after learning the difficult discriminations. Ongoing analyses assess whether individual neurons and CA1 population activity encode stimulus identity, discrimination difficulty, behavioral choice, and task outcome, and whether these representations change with expertise.

By combining behavioral measurements with longitudinal calcium imaging, this study aims to determine if CA1 representations evolve during visual perceptual learning. Characterizing experience-dependent changes in hippocampal activity may clarify how memory-related circuits interact with sensory and decision-making processes during the acquisition of fine perceptual discriminations.

Shaping Visual Features through Optogenetic Pairing in Injured Visual Cortex

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The precise timing between pre- and postsynaptic activity is a critical determinant of synaptic plasticity. Here, we investigated whether temporally precise visual-optogenetic pairing could selectively modulate cortical responses in the perilesional primary visual cortex (V1) of adult mice following focal lesion.

Adult mice expressing Channelrhodopsin-2 in V1 underwent a two-week conditioning protocol in awake head-fixed conditions. During training, one visual orientation was paired with optogenetic stimulation delivered at defined temporal delays relative to visually evoked cortical activity, while an orthogonal orientation presented without led stimulation served as an internal control. Visual Evoked Potential recordings revealed orientation-selective changes that critically depended on the temporal offset between visual and optogenetic activation. Pairing optogenetic stimulation with the peak of the visually evoked response enhanced responses to the paired orientation, particularly in deep cortical layers. In contrast, delivering optogenetic stimulation before visually evoked activity shifted cortical responses toward the unpaired orientation, demonstrating bidirectional modulation consistent with spike-timing-dependent plasticity mechanisms.

Control groups exposed to visual stimulation alone or to head-fixation without stimulation failed to show significant orientation-selective modulation.

Histological analysis further revealed alterations in excitatory and inhibitory synaptic markers within perilesional cortex.

Together, these findings demonstrate that temporally precise visual-optogenetic pairing can bidirectionally shape orientation-selective responses in injured visual cortex, highlighting temporal coding as a key mechanism for directing post-lesional cortical plasticity.

Motor Rhythm and Corticospinal Excitability during Action Observation: Evidence from an EEG-TMS study

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TMS studies have demonstrated that action observation (AO) facilitates corticospinal excitability (CSE), as reflected by increased motor-evoked potential (MEP) amplitude. At the same time, EEG studies have shown that AO is associated with a desynchronization of the sensorimotor mu rhythm, reflecting changes in the ongoing state of the motor system. Importantly, recent studies indicate that, at rest, when the mu rhythm is typically synchronized, MEP amplitude is influenced by the phase of motor oscillations at the time of TMS pulse delivery. This raises the question of whether similar oscillatory dynamics continue to shape corticospinal excitability also during AO, when the mu rhythm is desynchronized. Here, we conducted an EEG-TMS study to investigate phase- and power-dependent modulation of MEP amplitude during rest and AO. Twenty-seven healthy, right-handed participants underwent two randomized conditions: (1) observation of a black screen (REST) and (2) observation of reach-to-grasp actions (AO). In each condition, 440 TMS pulses were delivered over the left primary motor cortex while EMG and 64-channel EEG were recorded simultaneously. Our analysis reveals that, during REST, MEPs amplitude was significantly modulated by the mu-rhythm oscillation phase (with larger amplitude occurring during the EEG early rising phase) and this modulation remained stable across different levels of mu-rhythm power. Conversely, during AO, when the mu rhythm was desynchronized, the phase-dependent modulation disappeared, suggesting that the engagement of the premotor-parietal mirror network may overwrite the phase-dependency of corticospinal excitability observed at rest. However, when the ongoing mu rhythm remained synchronized despite action observation, the phase-dependent modulation re-emerged, reproducing the same phase relationship observed during rest. Overall, these findings suggest that the phase-dependent modulation of corticospinal excitability is not a fixed property of primary motor cortex activity but depends on the functional state of the broader motor network.

Combining TMS and Action Observation to probe motor-network integrity in presurgical glioma patients

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The observation of others' actions recruits the same fronto-parietal network involved in action execution. This phenomenon, known as the 'mirror mechanism' can be assessed in humans indirectly via transcranial magnetic stimulation (TMS) over the primary motor cortex, by measuring action observation (AO)-induced facilitation of corticospinal excitability. Clinically, the mirror mechanism has been applied in motor rehabilitation, where AO promotes cortical plasticity following peripheral damages or central lesions. A well-established literature has demonstrated that AO engages widespread sensorimotor areas, offering a tool to probe the functional integrity of patients with motor networks lesions. In this framework, patients with fronto-parietal glioblastoma represent a highly informative model for investigating how focal lesions affect the functional organization and connectivity of the motor system. We present a study aimed at introducing AO combined with TMS into the presurgical assessment of patients with glioma. Thirty patients were recruited preoperatively. Single-pulse TMS was delivered over the ipsilesional and controlesional primary motor cortices, while electromyography recorded motor-evoked potentials (MEPs) from three hand muscles involved in grasping. Fifteen MEPs were collected at rest and during the observation of reach-to-grasp actions (AO). Preliminary findings indicate that AO-induced facilitation distributes in the brain unevenly according to the tumor localization. Lesion-symptom mapping, diffusion tensor imaging, histological features and cortical dysconnectomics further representing the impact that individual tumor exerts onto the anatomo-functional properties of the motor network. Combining TMS and AO may provide an indirect proxy of motor network integrity, paving the way for future diagnostic approaches. Moreover, longitudinal post-operative subministration of AO during rehabilitative TMS could assess the motor regions responsiveness and potentially identify a biomarker of individual sensitivity to neuromodulatory interventions.

Spatiotemporal signatures of cortical modulation during action observation: a TMS-EEG study

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Since the first evidence of the mirror mechanism (MM) in humans, Transcranial Magnetic Stimulation (TMS) has become a key tool for probing corticospinal excitability during action observation (AO). However, conventional TMS mainly captures changes within primary motor cortex, providing only a partial view of how AO-related modulation unfolds across the sensorimotor system. Combining TMS with Electroencephalography (TMS-EEG) allows direct tracking of TMS-evoked cortical responses across the scalp with high temporal resolution. We stimulated the left dorsal premotor cortex, a key node of the MM, in 18 healthy volunteers while 64-channel EEG was continuously recorded. TMS pulses were delivered while participants observed reach-to-grasp actions, landscapes, or a black screen. Individual MRI-guided neuronavigation and real-time monitoring of TMS-evoked potentials (TEPs) were used to optimize stimulation and minimize artifacts. We first assessed cortical excitability over electrodes closest to the stimulation site. Local TEPs features (amplitude, latency) were significantly modulated during AO compared with control conditions across early (~50 ms), middle (~100 ms), and late (~200 ms) components. During AO, electrodes beneath the coil also showed a higher natural frequency, reflecting a shift in the dominant TMS-induced oscillatory activity within the first 100 ms. Topographic analysis (tANOVA, microstates) further revealed condition-dependent differences in scalp distribution temporally overlapping with the local amplitude effects, particularly in the middle latency component. Importantly, effective connectivity analysis revealed increased beta-band information flow from FC1 toward both ipsilateral C3 and contralateral FC2 during AO, specifically within the first 100 ms after TMS. These findings indicate that AO induces a distinct spatiotemporal reconfiguration of TMS-evoked cortical activity, affecting local responsiveness, scalp organization, oscillatory dynamics, and directed premotor-to-motor network interactions. TMS-EEG therefore offers a powerful framework to resolve when and how action-related activity propagates across the human motor network, providing a network-level characterization of the MM beyond corticospinal output alone.

5. Brain Plasticity, Stroke and Neuroinflammation

P32

Structural brain disconnection after ischemic stroke: from focal lesions to disrupted networks

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Ischemic stroke has the potential to damage not only the infarct core but also the axonal projections passing through it, disconnecting distant yet structurally intact brain regions. In humans, post-stroke deficits are thought to reflect such network-level disconnection in addition to lesion size, but the contribution of structural disconnection remains poorly characterized in preclinical models. Here, we aimed to quantify the whole-brain structural disconnection induced by a focal ischemic lesion, following distal middle cerebral artery occlusion (dMCAO), in adult C57BL6 mice.

At 30 days post-stroke, the lesion was mapped and reconstructed in three dimensions onto the Allen Mouse Brain Common Coordinate Framework (CCFv3) by using ABBA (Aligning Big Brains & Atlases). We then combined the lesion maps with the Allen Mouse Brain Connectivity Atlas (AMBCA; 498 anterograde tracer experiments) to estimate which brain regions and axonal projections were disrupted by the stroke. This approach allowed us to move beyond lesion area and identify the broader network of brain regions affected by the ischemic lesion. Preliminary analyses in a cohort of six mice revealed a consistent pattern of structural disconnection involving predominantly sensorimotor and thalamostriatal regions, including primary and secondary somatosensory and motor areas, as well as thalamic and striatal nuclei. Disconnection also extended through callosal pathways to homotopic regions in the contralateral hemisphere. These findings suggest that a relatively focal cortical lesion can produce widespread alterations in structural brain connectivity.

The next step will be to relate individual patterns of structural disconnection to behavioral performance assessed before and after stroke. Importantly, this study will establish a novel approach for characterizing stroke-induced structural disconnection in the dMCAO model, addressing a gap that has not yet been explored. Beyond ischemic stroke, the methodology could be extended to other models of focal brain injury, particularly in experimental settings where MRI-based assessment of structural connectivity is not available. More broadly, this work will

therefore provide a network-level framework for understanding how focal ischemic lesions alter brain organization and how these alterations may contribute to functional impairment and recovery.

Characterization of perilesional motor cortical activity after stroke in an adult mouse model

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We recorded local field potentials (LFPs) and multi-unit activity (MUA) from the motor cortex using a 32-channel bipolar depth electrode in a mouse model of ischaemic stroke. Stroke was induced by the distal middle cerebral artery occlusion (dMCAO) surgery technique, eliciting the generation of focal stroke involving the somatosensory cortices. Recordings were acquired during head-fixed locomotion on a running wheel at baseline and across multiple time points up to 30 days after stroke induction. Slow-wave events were detected in the 0.1–4 Hz LFP and characterized by their associated neuronal down-state, quantified independently as reductions in multi-unit firing rate and burst rate. For each event, we quantified low-frequency (1–30 Hz) and high-frequency (30–90 Hz) time–frequency power, together with the spectral composition of the event using FOOOF to separate periodic oscillatory activity from the aperiodic 1/f component. In parallel, we performed a movement-onset-locked analysis applying the same metrics around the initiation of locomotion, and quantified behavioral performances from the wheel sensor by measuring running distance, speed, and bout organization.

Results: Across the recovery period, the overall characteristics of slow-wave events remained largely stable, with no clear lesion-dependent gradient or evidence of systematic changes in down-state coupling between superficial and deep layers. The main effect observed was a state-dependent modulation in the number of slow-wave events associated with a significant firing-rate drop: these events longitudinally decreased during resting periods while becoming more frequent during locomotion. In contrast, the duration of slow-wave events increased over time in both behavioral states. Other spectral and neuronal activity measures showed only modest variations across recovery, without consistent depth-dependent or lesion-specific trends.

From neuroinflammation to behavior: uncovering microglia role in perinatal stroke

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Perinatal ischemic stroke, occurring between the 20th gestational week and the 28th postnatal day, affects about 1 in 2,300 live births and coincides with critical windows of brain development and heightened plasticity. Following the ischemic event, the injury induces neuronal death and triggers microglia, the resident immune cells in the brain. While microglia-driven inflammation is associated with detrimental outcomes in adulthood, their role in the developing brain after early-life injury remains poorly understood. To tackle this challenge, we induced a cortical lesion by permanent middle cerebral artery occlusion (MCAO) on postnatal day (P)14 and assessed spontaneous recovery by longitudinal behavioral testing (2-79 days post-injury). Stroke mice revealed long-lasting sensorimotor deficits in the grid walk test, associated with a core lesion volume of about 2.5 mm³ and a reduction in neuronal population (NeuN+ cells) in the perilesional area. To investigate microglial contribution, mice received the CSF1R inhibitor (PLX5622) via intraperitoneal injections, for 5 consecutive days after surgery, achieving 85% depletion of Iba1+ cells. Given the role of microglia in synaptic refinement, we therefore analyzed post-stroke induced reorganization by quantifying excitatory (vGLUT-PSD95) and inhibitory (vGAT-Gephyrin) markers together with parvalbumin-positive (PV+) interneurons and perineuronal nets (PNNs). In the peri-infarct cortex, stroke animals showed a significant reduction in PV+ cell density and WFA-labelled PNNs at D80 post-injury. These alterations, together with the reduction in inhibitory pre-synaptic puncta, are consistent with a long-lasting alteration in cortical circuit maturation and stabilization after the primary ischemic event. Notably, acute transient depletion of Iba1+ cells attenuated chronic sensorimotor deficits in the grid walk test, without affecting the final lesion volume, supporting a role for early microglia responses in shaping functional recovery after perinatal stroke. Finally, to integrate central and systemic inflammation, we collected peripheral blood from the same animals to correlate circulating inflammatory markers with microglial activity and functional outcomes. Altogether, our findings will shed light on microglia role in neuroinflammation, synaptic remodeling and neuroimmune crosstalk in the developing brain, providing a foundation for potential therapeutic strategies.

Temporal dynamics and relevance to rehabilitation by action observation treatment of blood biomarkers of brain injury and inflammatory in acute ischemic stroke

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Stroke is a leading cause of mortality worldwide and a major driver of long-term disability. Among the pathological processes triggered by cerebral ischemia, brain damage and inflammation are considered key determinants impacting on brain plasticity and on the possibility to recover. In a time and context dependent manner mediators released by damaged tissue may either support reparative mechanisms or exacerbate sustained inflammation, ultimately hindering brain plasticity and promoting further neuronal injury and cell death.

Two monocentric clinical studies enrolling ischemic stroke patients (~70) admitted to IRCCS Humanitas Research Hospital collected blood samples at 5, 30, and at follow up to measure levels of brain injury and inflammatory biomarkers by means of SIMOA and ELLA platforms. Patients received MRI scans for structural and functional parameters. Results were compared to age- and sex-matched healthy subjects. One cohort with impaired upper limb function received rehabilitation by Action Observation Treatment (AOT) by means of virtual reality.

Cross-cohort harmonization of measurements, with values from one cohort rescaled to the metric of the reference cohort, was undertaken in order to provide a common measurement scale. This improved comparability of measurements and facilitated the integration and direct comparison of findings across cohorts.

Biomarker trajectories were analyzed in relation to clinical scores and MRI-measures. Both cohorts showed similar biomarker dynamics, with levels significantly higher than healthy subjects (HS) at different time points, which tended to return to the levels of HS. Notably, biomarkers at 5 days post-stroke correlated strongly with lesion volume, enabled patient stratification by severity, and predicted rehabilitation outcomes 57 days after therapy.

Logistic analyses assessed their prognostic value for predicting recovery after rehabilitation. The impact of AOT on brain damage biomarkers suggest a potential measurable effect.

These findings support the utility of blood-based biomarkers in stratifying stroke patients and predicting rehabilitation outcomes. This offers promising insights into stroke severity through the measurement of secondary injury biomarkers at subacute time-points, which could be used as proxies to evaluate the recovery potential and the extent of brain plasticity.

Iron Accumulation triggers microglial paracrine senescence via EVs-SARM1-Dependent NAD⁺ axis

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Background. Microglia are essential for neuronal function, but in neurodegeneration they acquire senescence-associated phenotypes characterized by metabolic dysfunction, proinflammatory signaling, and increased Extracellular Vesicle (EV) release. Intracellular iron accumulation is a hallmark of aging and diseased brain, yet its role in driving microglial senescence remains unclear. My research focuses on understanding how iron-induced microglial dysfunction propagates to neighboring cells, both in vitro and in the mouse brain, and explores the underlying molecular mechanisms. **Methods.** Murine microglia were chronically exposed to iron (for 15 and 30 days) to model senescence. Cellular, transcriptomic, and metabolic alterations were assessed by immunofluorescence, WB, RNA-seq, and metabolomics. Paracrine effects were evaluated in healthy cells using non-contact co-cultures, senescent-derived EVs/soluble factors, and transplantation of iron-loaded senescent microglia into the mouse brain. **Results.** Chronic iron exposure induced a senescence-like phenotype in microglia, marked by proliferative arrest, increased SA- β -galactosidase activity, decreased NAD⁺ levels, and enhanced EV release. Notably, senescent microglia transferred senescence-associated features and NAD⁺ depletion to neighboring healthy cells, an effect prevented by inhibiting the activity of the NAD-consuming enzyme SARM1. Finally, EVs from senescent microglia directly stimulated recombinant SARM1 activity. **Conclusion.** These findings indicate that iron-loaded microglia propagate senescence through SARM1-dependent NAD⁺ depletion and that EV-associated factors contribute to this

process. Therefore, EVs-SARM1-NAD⁺ axis may represent a novel pathological mechanism and a promising therapeutic target to mitigate neurodegeneration.

Studying lifestyle impact on synapses using proximity labeling-based proteomics

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A healthy lifestyle is the first step towards preserving the physiological homeostasis of the central nervous system. Indeed, moderate physical exercise, coupled with cognitive stimulation and social interaction, preserves learning and memory abilities throughout life, and prevents the onset of a variety of pathologies, including neurodegenerative diseases.

The functional, cellular, and molecular mechanisms underlying these benefits can be studied in rodent models by means of environmental enrichment (EE), an experimental paradigm based on providing animals with free access to voluntary physical exercise, along with enhanced sensory stimulation and social interaction.

Reproducing the effect of EE using appropriate drugs holds great translational potential but requires a complete picture of the molecular mediators EE leverages on, and this has not been drawn so far.

As a step towards filling this gap, we set up a genetically encoded probe, based on the proximity labeling approach, to isolate the proteome of excitatory synaptic clefts.

The miniTurboID biotin ligase was fused to the Lrrtm1 protein to achieve localization at excitatory synapses. This construct was placed under DIO control to trigger expression only upon Cre recombinase-mediated activation. Moreover, the use of tamoxifen-dependent Cre-ERT2 allowed to restrict the time window for expression to the desired interval.

After tests in primary neuronal cultures, the constructs were packed in Adeno Associated Viral vectors (AAVs) for in vivo transduction. Expression of TurboID-Lrrtm1 in the hippocampi of mice reared in EE and standard rearing controls enabled retrieval of biotin-labeled synaptic proteins via immunoprecipitation. Samples were subsequently processed for mass spectrometry, followed by comparative bioinformatic analysis.

Our datasets of differentially expressed proteins will be further analyzed to determine the key molecular effectors responsible for the positive effect of lifestyle on the healthy and diseases brain, with the final goal of identifying novel druggable targets.

Persistent multiscale cortical reorganization following peripheral sensorimotor blockade

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Transient perturbations of synaptic activity can induce adaptive changes throughout the nervous system; however, whether a localized and reversible peripheral blockade is sufficient to drive persistent brain-wide reorganization remains unclear. Here, we addressed this question using a single injection of botulinum neurotoxin type A1 (BoNT/A1) into the mouse whisker pad, a model of transient peripheral synaptic blockade. In addition to blocking peripheral neurotransmission, BoNT/A1 undergoes retrograde axonal transport and transiently silences a restricted population of second-order central synapses, providing a unique model to investigate how localized circuit perturbations propagate across distributed neural networks. Although peripheral sensorimotor function recovered within approximately 10 days, BoNT/A1 induced long-lasting changes throughout the brain. Thirty days after treatment, dendritic spine density was significantly reduced in motor cortical regions, while longitudinal two-photon imaging revealed enhanced spine elimination and progressive structural remodeling beginning at 15 days after injection. These structural changes were accompanied by persistent functional deficits. Treated mice displayed impaired performance in a whisker-dependent sensorimotor task well beyond peripheral recovery, together with reduced c-Fos activation across cortical and subcortical regions. Wide-field calcium imaging further revealed long-lasting alterations in intra- and interhemispheric functional connectivity. Together, these findings demonstrate that a transient and spatially restricted synaptic blockade is sufficient to trigger enduring multiscale reorganization of brain circuits, linking structural remodeling, network reconfiguration, and persistent behavioral impairment.

6. Neuropsychiatric Disorders: Neuropathogenesis and Therapeutic Agents

P39

A dual serotonergic approach for reducing methamphetamine-induced alterations on schizophrenia-associated behaviors in male and female rats

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Methamphetamine (METH) is a potent psychostimulant that produces profound alterations in brain functions and can induce psychosis among recreational and chronic users, with some users developing a persistent psychotic syndrome that shows similarities to schizophrenia. Notably, individuals with METH-related disorders have a higher risk of schizophrenia than those with other drug use disorders, with the exception of cannabis use disorders. While METH exerts its reinforcing effects largely through dopaminergic mechanisms within the mesocorticolimbic system, converging evidence indicates a critical modulatory role of serotonin (5-hydroxytryptamine, 5-HT) receptors in both the acute and long-term behavioral consequences of psychostimulant exposure. In particular, 5-HT_{2B} receptors have been implicated in the facilitation of amphetamine- and METH-induced monoamine release, locomotor activation, and reinforcing effects, while 5-HT_{1A} receptors play a central role in the regulation of mood, anxiety, stress responsivity, and cognitive flexibility. Recently, we found that new dual serotonergic compound DDD-024, acting as 5-HT_{2B} receptor antagonist and 5-HT_{1A} receptor agonist, is able to dose-dependently reduce METH-related drug-taking and drug-seeking behaviors in rats, in a sex-dependent manner and without compromising natural reward processing or safety.

In this follow-up study, we assessed in rats of both sexes the effects of the pretreatment with DDD-024 on (i) sensorimotor gating functions (pre-pulse inhibition test), (ii) working memory (novel object recognition test), (iii) social behaviors (social interaction test) and (iv) anxiety-like states (elevated plus maze test, marble burying test). Findings indicated that, when tested at the same intraperitoneal (i.p.) doses known to be effective on METH intake and relapse (i.e., doses ranging from 4 to 10 mg/kg), DDD-024 does not exert strong significant effects on schizophrenia-associated behaviors. Yet, when tested on acute METH-induced behavioral alterations, we found that DDD-024 is able to significantly reduce METH's effects in a dose-dependent and sex-specific

manner. These findings have clinical implications since they suggest that DDD-024, which per se does not significantly affect sensorimotor, cognitive and social skills or anxiety-like behaviors states, could be helpful in ameliorating behaviors that are commonly altered in schizophrenia.

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Serotonergic mechanisms regulating cognitive skills and plasma corticosterone levels in methamphetamine-treated rats

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Cognitive dysfunction is a yet scarcely controlled feature of neuropsychiatric disorders, including drug addiction, with a negative impact on quality of life and psychosocial functioning. Methamphetamine (METH), among the most abused drugs worldwide, is a psychostimulant with several neuropsychiatric symptoms including cognitive impairment. No effective medication is available for managing METH addiction. Hence, the discovery of a safe and effective therapeutic is a critical unmet medical need for METH addiction. METH promotes the release of dopamine, serotonin (5-HT) and norepinephrine in several brain areas; mesolimbic dopaminergic activity modulates reward-oriented behavior and is regulated (among others) by 5-HT receptors. The 5-HT_{1A} receptor is the most widespread of all 5-HT receptor subtypes and has been involved in virtually all of the biochemical and behavioral effects of METH abuse including anxiety, depression, memory impairment, and cognitive dysfunction. Here we tested the hypothesis that the new compound DDD-024 (Daya Drug Discovery, USA), which possesses both 5-HT_{1A} agonist and 5-HT_{2B} antagonist properties, would represent a therapeutic option for METH-induced cognitive impairment.

Young adult rats underwent chronic METH treatment for 10 days before starting DDD-024 administration (10 mg/kg for males, 7 mg/kg for females) on day 11, followed by behavioral testing in the Morris water maze test on day 12. Spatial learning was assessed over 4 days of training (days 12 to 15 of treatment), across repeated trials in which all male and female rats learned to locate the hidden platform, irrespective of drug treatment. Memory performance was assessed on day 16 in a probe test; in males, no differences in average proximity to the platform area were observed, with all the groups remembering the correct platform location. By contrast, METH-treated females showed impaired memory, an effect not rescued by DDD-024 treatment. We next evaluated the effect of chronic METH and DDD-024 treatments on plasma corticosterone (CORT) levels in the same rats. METH increased CORT levels in both males and females and such effect was not blocked by DDD-024 treatment.

Overall, the dual 5-HT_{1A}/5-HT_{2B} compound DDD-024, which per se does not affect cognitive skills in the Morris water maze test, fails to rescue METH-induced memory impairment and altered CORT levels. Thus, other mechanisms may be involved in such effects.

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Investigating the role of endosomal signaling in the neuroplastic effect of psilocybin

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Psilocybin, the compound found in “magic mushrooms” and prodrug of psilocin, has gained attention as a promising therapeutic agent for the treatment of numerous psychiatric diseases, producing rapid and long-lasting clinical benefits after a single administration. These therapeutic effects are thought to rely on its ability to enhance structural and functional neuroplasticity, particularly within the prefrontal cortex and the hippocampus. Although psilocin is an agonist of the serotonin receptor 5-HT_{2A} (5-HT_{2AR}), how its activation leads to long-term plastic remodeling remains unclear. Recent evidence suggests that psilocin may diffuse into cells and promote neuroplasticity by activating intracellular 5-HT_{2AR}s, rather than through conventional signaling at the plasma membrane. An intriguing hypothesis is that, once internalized in the cell, psilocin could be protonated and retained inside intracellular acidic compartments, leading to its accumulation and explaining its prolonged action. To test this hypothesis, we generated two innovative chemical tools: a fluorescent-modified psilocin derivative, that we use to monitor psilocin intracellular distribution in real-time by live-cell imaging, and a photoaffinity-labeling-modified psilocin, which we coupled with click chemistry to precisely pinpoint psilocin subcellular interactome in a specific time-frame. By using advanced imaging techniques both *in vitro* and *ex vivo*, we demonstrate that psilocin rapidly diffuses into neurons and accumulates in acidic compartments in a pH-dependent manner. Furthermore, photoaffinity labeling confirms that psilocin localizes within endomembranes, pointing to a direct interaction with subcellular receptors on endosomes. Remarkably, a 20-minute-long stimulation with psilocin is sufficient to trigger neuronal growth, observed 72h later, suggesting that the signaling cascade is sustained even when the drug is removed from the medium and that retention within the endosomal lumen might be functional to sustained interaction with endosomal receptors. Our approach offers a novel and powerful strategy to elucidate the role of intracellular acidic compartments in the pharmacology of neuroplastogens. In addition to addressing a key open question regarding the neuroplastic potential of 5-HT_{2AR} agonists, this work lays the foundation for the rational design of next-generation serotonergic agents that leverage intracellular drug retention to achieve more durable neuroplastic outcomes.

7. Cancer and Radiation Biology

P42

In vitro and in vivo models to assess the impact of ionizing radiation on calcium dynamics to counteract Glioblastoma

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Glioblastoma (GB) is the most aggressive primary brain tumor and is characterized by massive infiltration of the surrounding parenchyma. Gliomas establish functional networks that rely on calcium (Ca^{2+}) signaling to promote proliferation, invasion and therapeutic resistance, highlighting Ca^{2+} dynamics as a potential therapeutic target. FLASH radiotherapy (FLASH-RT), which delivers radiation at ultra-high dose rates (UHDR), has emerged as a promising approach for brain malignancies, capable of limiting the damage to healthy tissues (FLASH effect), compared to Conventional Radiotherapy (CONV-RT). Ionizing radiation is known to perturb Ca^{2+} activity, yet the effects on these dynamics remain poorly understood, particularly in vivo. In this study, we employed an electron LINear ACcelerator (LINAC), able of switching between UHDR and CONV-RT. Murine GL261 and CT2A glioma cells expressing the GCaMP6s calcium sensor were orthotopically implanted in mice, and Ca^{2+} activity is longitudinally monitored by Two-Photon imaging before and after RT. Clonogenic assays revealed different dose-dependent responses, with GCaMP6s-GL261 cells being more sensitivity to FLASH-RT. Preliminary in vivo experiments showed that both irradiation modes significantly delayed tumor growth compared to non-

irradiated mice, with similar anti-tumor efficacy. Furthermore, GCaMP6s-GL261 cells appear undergoing apoptotic pathways, reflecting the technical challenges in acquiring in vivo calcium dynamics post RT. This is the first study that compares tumor-associated Ca^{2+} responses in in vivo GB models following CONV-, FLASH- and Minibeam-RT (MBRT). I aim to investigate in real time the role of Ca^{2+} signaling in radiation response and evaluate whether the spatially fractionated irradiation MBRT may further improve healthy tissue sparing while maintaining therapeutic efficacy, thus resulting in a safer clinical alternative for cancer treatment, particularly in sensitive anatomical sites like the brain.

Phase II study of FLASH versus conventional radiotherapy's Abscopal & Bystander Local Effects (FLASH-ABLE)

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FLASH radiotherapy (FLASH-RT), delivered at ultra-high dose rates (>40 Gy/s), has emerged as a promising strategy capable of reducing normal tissue toxicity while preserving tumor control compared to conventional radiotherapy (CONV-RT). However, the mechanisms underlying its systemic immune modulation, including bystander and abscopal effects, remain largely unexplored in patients. The project aims to characterize and compare the local and systemic immune responses elicited by FLASH- and CONV-RT in patients with non-melanoma skin cancer (NMSC) enrolled in the randomized phase II ULISSE clinical trial. Forty patients (20 FLASH-RT, 20 CONV-RT) will receive a single 22.5 Gy fraction and will undergo longitudinal blood sampling (baseline, 2 weeks, 3 and 6 months) together with skin biopsies (3 and 6 months). We will investigate: (i) peripheral immune cell phenotyping by multiparameter flow cytometry, including T-cell subsets, activation/exhaustion markers, NK cells, B cells and regulatory T cells; (ii) circulating cytokine, chemokine and Granzyme B profiling using multiplex immunoassays; (iii) circulating free DNA analysis by digital PCR for treatment monitoring; and (iv) spatial immune characterization of irradiated tissues by multiplex immunofluorescence and confocal microscopy. Integrative statistical analyses will correlate immune signatures with treatment modality, clinical characteristics and therapeutic response to identify biomarkers associated with FLASH-specific immune modulation. The project builds upon extensive preclinical expertise demonstrating distinct oxidative, mitochondrial and metabolic responses induced by FLASH-RT in tumor models. Multiparameter flow cytometry antibody panels, immunofluorescence staining protocols, confocal imaging procedures and data analysis pipelines have been fully optimized using peripheral blood and tissue samples from a healthy human control subject, confirming technical

feasibility, assay reproducibility and readiness for clinical sample analysis. FLASH-ABLE will provide the first comprehensive clinical characterization of immune bystander and abscopal responses following FLASH-RT in NMSC patients. The identification of treatment-specific immune biomarkers may support the rational integration of FLASH radiotherapy with immunotherapeutic approaches and contribute to the development of more effective, personalized radiotherapy strategies with improved efficacy and reduced toxicity.

8. New Tools in Neuroscience

P44

A Self-Base Editing Strategy to Limit Base Editor Activity Following AAV Delivery

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CRISPR-based adenine base editing (ABE) represents a promising therapeutic strategy for the correction of pathogenic missense variants, particularly G to A transitions, which account for the majority of disease-causing mutations. By directly restoring the endogenous sequence with high efficiency and precision, base editing has the potential to overcome the limitations of conventional gene supplementation therapies.

A major challenge for the clinical translation of base editing is achieving transient editor activity following in vivo delivery. While AAVs enable efficient transduction of the central nervous system, they also drive prolonged expression of the editor, increasing the risk of off-target editing as well as immune responses. Current strategies to limit editor activity often require exogenous molecules or large regulatory elements, limiting their compatibility with the restricted packaging capacity of AAV vectors.

This project aims to develop a compact, autonomous and tunable self-inactivation strategy based on self-base editing. We are exploiting small artificial introns within the nCas9 coding sequence in which the splice acceptor is disrupted by the editor itself. Following successful editing, correct mRNA splicing is abolished, leading to termination of Cas9 expression after a defined editing window. Importantly, the kinetics of editor shutdown can be modulated through the efficiency of the self-targeting sgRNA, allowing precise temporal control without external factors. Preliminary results showed that disruption of the splice acceptor completely abolishes transgene expression, validating the proposed mechanism. Then, we selected two self-targeting sgRNAs with distinct editing efficiencies, enabling tunable self-inactivation, and demonstrated that an optimized artificial intron preserves Cas9 expression and editing activity.

Ongoing studies are evaluating the impact of different self-editing kinetics on therapeutic editing efficiency and long-term editor expression. The optimal configuration will then be assessed for its ability to reduce off-target editing in a disease-relevant model and implemented using a compact Cas9 orthologue to generate an all-in-one AAV-compatible therapeutic platform. This work will establish a broadly applicable self-limiting base editing technology that enhances

the safety of AAV-mediated genome editing, providing a versatile platform for the treatment of neurodevelopmental disorders.