20th Meeting of the Spanish Society for Neuroscience

3-5 september 2025

Las Palmas de Gran Canaria

ABSTRACT BOOK



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SOCIEDAD ESPAÑOLA DE NEUROCIENCIA

INDEX

PLENARY LECTURES 3

SIMPOSIUM 8

ABSTRACTS 48

Developmental Neurobiology 49
Neuronal excitability, Synapses and Glia: Cellular mechanisms 94
Sensory and Motor Systems 179
Cognitive and Behavioral Neuroscience 210
Theoretical and Computational Neuroscience 277
Disorders, Nervous System repair and Healthy Aging 295
Homeostatic and Neuroendocrine Systems 484
Circuit Dynamics and Oscillations 493
New Methods and Technologies 508
History, Education and Ethics 542
General Neuroscience Topics 545

PLENARY LECTURES

The regulation of neural stem cell quiescence by a physical niche

Isabel Fariñas. Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Departamento de Biología Celular, Biología Funcional y Antropología Física, Instituto de Biotecnología y Biomedicina (BioTecMed), Universidad de Valencia

Details

New neurons for highly plastic olfactory circuits are produced in the subependymal zone (SEZ) of the adult mammalian brain. Neural stem cells (NSCs) in this niche have access to a wide range of regulatory signals that promote continuous lifelong neurogenesis while preserving the stem cell pool. NSCs derive from radial glial cells, which are the primary embryonic progenitor type in the vertebrate brain, and inherit from them part of their transcriptional program, a bipolar elongated morphology with apico-basal polarity that allows for unique interactions with neighboring cell types, and markers associated with the astrocytic lineage. In contrast to their fetal counterparts, most adult NSCs remain in a quiescent state under physiological conditions. It is now widely accepted that NSCs in the SEZ exist in at least three states: quiescent (q), quiescent but prone to activation or primed (p), and activated (a), each characterized by unique and distinct transcriptional profiles. Transitions between states likely involve significant changes in cellular physiology tightly regulated by both intrinsic and extrinsic factors. We have found that entry into quiescence is associated with the deposition of specific extracellular matrix components and that adhesion to the matrix produced in response to pro-quiescent signals alone can induced a quiescent-like state in proliferative NSCs. This entry into quiescence depends on the RhoA-associated kinase ROCK and yes-associated protein (YAP) transcriptional activity. YAP/TAZ deletion in NSCs leads to the loss of ECM deposition and quiescence in vivo suggesting that they regulate the physical niche and a quiescence-associated gene expression program in response to mechanical cues.

Tripartite Synapses: Astrocyte regulation of synaptic function, network activity and animal behavior

Alfonso Araque, Dept. of Neuroscience, University of Minnesota

Details

Astrocytes, a major type of glial cells, are recognized as key supportive elements in neuronal function, providing structural and metabolic support for neurons, and controlling brain homeostasis. Historically, they were overlooked as being active players in cellular processes underlying brain function. However, accumulating evidence indicate that astrocytes and neurons establish bidirectional communication. Astrocytes respond to synaptically-released neurotransmitters and, in turn, release gliotransmitters that influence neuronal and synaptic activity. This evidence has led to the establishment of the tripartite synapse concept, a novel view of synaptic physiology in which astrocytes are integral elements involved in synaptic function.

I will present current evidence regarding the mechanisms and functional consequences of the bidirectional astrocyte-neuron signaling at synaptic, circuit and behavioral levels. I will present results indicating that astrocytes spatially and temporally integrate neuromodulatory signals to regulate neural network function, suggesting that astrocytes sense the environmental conditions, including neuromodulators and network function states. Hence, astrocytes sense ongoing neuronal activity along with neuromodulators and, acting as neuromodulators, inform the neurons about the state of the internal system and the external world. In summary, I will discuss how current evidence that has led to a paradigm shift in our understanding of the cellular basis of brain function, which would result not solely from the neuronal activity, but from the coordinated activity of astrocytes and neurons.

Oligodendrocytes in brain energy metabolism and Alzheimer's disease

Klaus-Armin Nave

Details

We found that oligodendrocytes, best known for making myelin and allowing fast saltatory impulse propagation in the CNS, also provide glycolysis products as metabolic support for fast spiking axons. Moreover, in white matter tracts, the myelin sheath is a dynamic compartment that continuously turns over. Thus, when glucose is limiting, myelinating oligodendrocytes can rapidly metabolize myelin-derived fatty acids to meet their own energy needs for survival and to prevent irreversible axon degeneration. Interestingly, oligodendrocytes and neurons both express and process the amyloid precursor protein (APP), but Aβ peptides that are generated within oligodendrocytes contribute largely to the cortical and not the white matter plaque load in Alzheimer's disease (AD) mouse models. This raises the question how oligodendroglial Aβ is reaching neuronal plaques. In the aging brain, there is a gradual decline of myelin structural integrity, which is likely to affect myelin turnover and the metabolic coupling between oligodendrocytes and axons. This could be a risk factor for the onset of AD, because myelin dysfunction acts as a driver of amyloid plaque deposition in mice. This is in part due to the inhibition of microglia in phagocytosing Aβ, which also identifies a new therapeutic target for delaying the onset of AD.

Beyond Neuroimmune Communication: Messengers and Cellular Targets

Carmen Guaza, Instituto Cajal CSIC

Details

The interest in the functional interactions between the Nervous System and the Immune System, without overlooking the Neuroendocrine System, has shaped my scientific career from an integrative perspective in both physiological and pathological processes. Immune messengers, such as cytokines and chemokines are not only mediators of neuroinflammation but also essential for the protection and repair of the CNS. In multiple sclerosis (MS), the pathology on which we have focused through animal models, cell models and in MS itself we have identified new therapeutic targets in an effort to slow its progression. We found that the endogenous cannabinoid system plays a protective role in brain-immune cross-communication, targeting brain endothelial cells, microglia, astrocytes, oligodendrocytes, and their progenitors. The ability of microglia to acquire various activation states, -crucial for maintaining homeostasis, inflammation, and repair- is modulated by endocannabinoid signaling (eCBSS). This signaling system i) restores the inhibitory immune checkpoint CD200-CD200R which is key in neuron-microglia interaction and is disrupted at certain stages of MS ii) enhances the removal of myelin debris by microglía, thereby promoting the differentiation of oligodendrocyte progenitors cells (OPCs) and leading to remyelination in a viral model of MS iii) reduces proteoglycan accumulation and astrogliosis around plaques, facilitating remyelination and functional recovery in mice. In recent years, we have also become interested in the gut-brain axis and the characterization of the microbial profile in MS models.



INDEX

SYMPOSIUM 01

Exploring the neural substrates of number sense: a perspective on genetics, behaviour and neural circuity

MAIN TOPIC: This symposium will address a fundamental yet underexplored area of neuroscience: the genetic and neural mechanisms that support our ability to perceive and estimate numbers. This capacity, known as “number sense,” relies on an evolutionarily conserved mechanism found across all vertebrates. This non-symbolic ability is postulated to operate through two distinct systems: the “object tracking system”, which accurately represents small quantities, and the “approximate number system”, enabling the rough estimation of larger numerosities without the need for counting.

Despite their critical role in cognition and relevance to neurodevelopmental disorders, the genetic and neural basis of number sense are still unclear. Dyscalculia, a congenital impairment in number/arithmetic learning, is believed to have a genetic component, yet the specific genes involved remain unknown. Recent advances in animal models provide novel opportunities to decipher the genetic and neural circuits underlying this ability.

This symposium, which will be guided by SENC’s code of conduct, is designed to fill this gap. First, Dr Torres-Pérez will discuss how genes associated with Williams syndrome, a rare multigenic condition, influence numerosity using zebrafish models. Prof Caroline Brennan will follow this research by assessing the neural circuits and genetic variants involved in quantity estimation, offering insights into cognitive impairments. Dr Zanon will continue with an evolutionary perspective, illustrating how numerical cognition and its neural underpinnings have developed across species. Dr Miletto-Petrazzini will conclude by examining the role of brain lateralisation in number sense, to reveal how numerical abilities are organised in vertebrate brains.

By bringing together cutting-edge research on the genetic, behavioural, and neural dimensions of number sense, this symposium will significantly advance our understanding of cognition and its dysfunction across species. This symposium will interest neuroscientists in cognitive, developmental, and comparative neuroscience, as well as those studying genetics and neurodevelopmental disorders.

OVERVIEW OF THE SYMPOSIUM: This symposium delves into a rapidly advancing area of cognitive neuroscience that remains underexplored: Numerical cognition or “Number sense”, which refers to the innate ability to perceive and estimate quantities. This fundamental cognitive skill relies on an evolutionary conserved mechanism in all vertebrates. This symposium will uniquely combine insights from genetics, neurobiology, and comparative psychology to uncover the biological underpinnings of this vital cognitive process.

SPEAKER #1

- Full name and affiliation: Jose Vicente Torres Pérez; Department of Cellular Biology, Functional Biology and Physical Anthropology, University of Valencia, Spain

-Recent publications of the speaker related to the topic of the symposium:

1. Sheardown, E., Torres-Perez, J. V., Anagianni, S., Fraser, S. E., Vallortigara, G., Butterworth, B., Miletto-Petrazzini, M. E., & Brennan, C. H. (2022). Characterizing ontogeny of quantity discrimination in zebrafish. Proceedings. Biological sciences, 289(1968), 20212544. https://doi.org/10.1098/rspb.2021.2544

2. Torres-Pérez, J. V., Anagianni, S., Mech, A. M., Havelange, W., García-González, J., Fraser, S. E., Vallortigara, G., & Brennan, C. H. (2022). baz1b loss-of-function in zebrafish produces phenotypic alterations consistent with the domestication syndrome. iScience, 26(1), 105704. https://doi.org/10.1016/j.isci.2022.105704

3. Sheardown, E., Mech, A. M., Petrazzini, M. E. M., Leggieri, A., Gidziela, A., Hosseinian, S., Sealy, I. M., Torres-Perez, J. V., Busch-Nentwich, E. M., Malanchini, M., & Brennan, C. H. (2022). Translational relevance of forward genetic screens in animal models for the study of psychiatric disease. Neuroscience and biobehavioral reviews, 135, 104559. https://doi.org/10.1016/j.neubiorev.2022.104559

- Relevance for the symposium:

Dr Torres-Pérez (ORCID: 0000-0001-6390-9690) is a Ramón y Cajal fellow at the University of Valencia and a member of SENC. He will serve as chairperson for the proposed symposium. Dr Torres-Pérez has made significant contributions to understand the genetic and epigenetic mechanisms underlying neural development, particularly in the context of neurodevelopmental and neurodegenerative disorders. His research aligns with the symposium's focus on the neurobiological and genetic basis of numerosity, as he uses zebrafish as a model organism to investigate the role of specific genes in cognitive functions such as quantity discrimination.

Dr Torres-Pérez's work on Williams syndrome (WS) is particularly relevant. His use of CRISPR-generated zebrafish mutants to study genes affected in WS provides crucial insights into how these genes impact numerosity and broader cognitive functions. His recent studies have demonstrated that zebrafish with loss-of-function mutations for WS-related genes show impairments in discriminating large numerosities, reflecting the altered number cognition seen in humans with WS. These findings bridge the gap between genetic mutations and behavioural outcomes, reinforcing the zebrafish model's relevance for understanding human cognitive conditions like dyscalculia.

Given his cutting-edge research on the genetic and epigenetic regulation of cognitive traits using zebrafish, Dr Torres-Pérez's talk will provide crucial insights into how specific genes shape numerosity and related cognitive functions. His multidisciplinary approach, combining CRISPR technology with behavioural assays, places him at the forefront of neurobiological research, making him an invaluable addition to the symposium.

Dr Torres-Pérez talk will cover concepts from his first-author research currently under evaluation at PNAS (Proceedings of the National Academy of Sciences of the United States of America):

Torres-Perez, J. V., Anagianni, S., Sheardown, E., Miletto- Petrazzini, M. E., Fraser, S., Butterworth, B., Vallortigara, G., Brennan, C. H. Lack of Williams syndrome-associated genes alters quantity discrimination in zebrafish. Manuscript ID: 2024-22138

SPEAKER #2

- Full name and affiliation: Caroline H Brennan; School of Biological and Behavioural Sciences, Queen Mary University of London, United Kingdom

- Recent publications of the speaker related to the topic of the symposium:

1. Messina, A., Potrich, D., Perrino, M., Sheardown, E., Miletto Petrazzini, M. E., Luu, P., Nadtochiy, A., Truong, T. V., Sovrano, V. A., Fraser, S. E., Brennan, C. H., & Vallortigara, G. (2022). Quantity as a Fish Views It: Behavior and Neurobiology. Frontiers in neuroanatomy, 16, 943504. https://doi.org/10.3389/fnana.2022.943504

2. Messina, A., Potrich, D., Schiona, I., Sovrano, V. A., Fraser, S. E., Brennan, C. H., & Vallortigara, G. (2022). Neurons in the Dorso-Central Division of Zebrafish Pallium Respond to Change in Visual Numerosity. Cerebral cortex (New York, N.Y.: 1991), 32(2), 418–428. https://doi.org/10.1093/cercor/bhab218

3. Miletto Petrazzini, M. E., & Brennan, C. H. (2020). Application of an abstract concept across magnitude dimensions by fish. Scientific reports, 10(1), 16935. https://doi.org/10.1038/s41598-020-74037-5

- Relevance for the symposium:

Professor Caroline Brennan (ORCID: 0000-0002-4169-4083) has a prominent role in the field of molecular genetics. Professor Brennan's research focuses on integrating molecular genetics, behavioural analysis, and comparative psychology to investigate complex cognitive and psychiatric disorders. Her innovative work using zebrafish as a model to understand the genetic and neurobiological underpinnings of cognitive functions makes her an ideal candidate for this symposium.

Professor Brennan's research on creating CRISPR mutants in zebrafish to explore candidate genes for numerosity, especially in the context of Fragile X syndrome and related disorders, offers significant potential for advancing our understanding of the genetic basis of numerical cognition. This line of research, which bridges molecular genetics and behavioural neuroscience, is critical for uncovering how genetic mutations affect cognitive abilities and their developmental trajectories.

Her studies have identified brain regions in zebrafish that respond selectively to changes in numerosity and have highlighted the evolutionary conservation of magnitude cognition circuits. By using zebrafish as a model, she has shed light on the molecular and neural processes involved in quantity representation, offering valuable insights into how these processes may relate to genetic vulnerabilities associated with disorders like Fragile X. Additionally, her research into the ontogeny of number cognition and its interplay with attention and memory-related mechanisms reinforces the importance of zebrafish as a model for studying both typical and atypical cognitive development.

Professor Brennan's expertise in both molecular genetics and behavioural neuroscience, coupled with her focus on numerosity and psychiatric disorders, positions her as a key contributor to discussions on the genetic and neural bases of cognition, making her an invaluable addition to the symposium.

SPEAKER #3

- Full name and affiliation: Mirko Zanon; Centre for Mind/Brain Sciences, University of Trento, Italy; and Translational Imaging Center, Michelson Center for Convergent Bioscience, University of Southern California, Los Angeles, USA

- Recent publications of the speaker related to the topic of the symposium:

1. Kobylkov, D., Mayer, U., Zanon, M., & Vallortigara, G. (2022). Number neurons in the nidopallium of young domestic chicks. Proceedings of the National Academy of Sciences of the United States of America, 119(32), e2201039119. https://doi.org/10.1073/pnas.2201039119

2. Zanon, M., Potrich, D., Bortot, M., & Vallortigara, G. (2022). Towards a standardization of non-symbolic numerical experiments: GeNEsIS, a flexible and user-friendly tool to generate controlled stimuli. Behavior Research Methods, 54(1), 146-157.

3. Adam, E., Zanon, M., Messina, A., & Vallortigara, G. (2024). Looks like home: numerosity, but not spatial frequency guides preference in zebrafish larvae (Danio rerio). Animal cognition, 27(1), 53. https://doi.org/10.1007/s10071-024-01888-0

- Relevance for the symposium:

Dr Mirko Zanon (ORCID: 0000-0003-4062-1496) is a postdoctoral research fellow with an interdisciplinary background in physics and neuroscience, which equips him with a unique skill set, allowing him to bridge fundamental principles from physics with advanced neural circuit modeling.

Mirko's recent work has made significant contributions to understanding numerical cognition across species, from chicks to fish. He identified number neurons in the caudal nidopallium of young chicks, analogous to the mammalian prefrontal cortex, suggesting that number perception may be an inborn trait of the vertebrate brain. His research on archerfish showed that non-human animals can abstract numerical information independent of physical attributes, offering new insights into fish cognition. His studies with zebrafish larvae further confirmed their reliance on numerical abilities rather than lower-level visual cues to distinguish different numerosities.

Mirko's cutting-edge application of two-photon fluorescence light sheet microscopy to track neuronal activity in larval zebrafish (Luu, P., Nadtochiy, A., Zanon, M., Moreno, N., Messina, A., Petrazzini, M. E. M., Torres Perez, J. V., Keomanee-Dizon, K., Jones, M., Brennan, C. H., Vallortigara, G., Fraser, S. E., & Truong, T. V. (2024). Neural Basis of Number Sense in Larval Zebrafish. bioRxiv : the preprint server for biology, 2024.08.30.610552. https://doi.org/10.1101/2024.08.30.610552.) represents a significant technical advancement in neuroscience. His ability to map number-selective neurons throughout the brain and use machine learning to predict stimuli based on

neural activity exemplifies his innovative approach to study neural circuits underlying number sense. This research also offered the first evidence of how these neurons develop with age.

Overall, Dr Zanon's expertise and pioneering research on neural circuits and numerical cognition across species make him an ideal addition to our symposium proposal. His interdisciplinary approach would attract those looking to bridge biology, computation, and behaviour in the context of cognition.

SPEAKER #4

- Full name and affiliation: Maria Elena Miletto-Petrazzini; Department of General Psychology, University of Padova, Italy

- Recent publications of the speaker related to the topic of the symposium:

1. Miletto Petrazzini, M. E., Sovrano, V. A., Vallortigara, G., & Messina, A. (2020). Brain and behavioral asymmetry: a lesson from fish. Frontiers in Neuroanatomy, 14, 11. https://doi.org/10.3389/fnana.2020.00011.

2. Miletto Petrazzini, M. E., Gambaretto, L., Dadda, M., Brennan, C., & Agrillo, C. (2021). Are cerebral and behavioural lateralization related to anxiety-like traits in the animal model zebrafish (Danio rerio)?. Laterality, 26(1-2), 144–162. https://doi.org/10.1080/1357650X.2020.1854280

3. Agrillo, C., & Miletto Petrazzini, M. E. (2021). Numerical competence in fish. In A. B. Kaufman, J. Call, & J. C. Kaufman (Eds.), The Cambridge handbook of animal cognition (pp. 580–601). Cambridge University Press. https://doi.org/10.1017/9781108564113.031

- Relevance for the symposium:

Dr Miletto-Petrazzini's work (ORCID: 0000-0002-5204-5863) focuses on cognitive and perceptual systems in vertebrates. Dr Miletto-Petrazzini has explored numerical abilities and brain lateralisation using zebrafish as a model species, integrating behavioural observations, neural activity recordings, and molecular analysis.

Her research into brain lateralisation, particularly its implications for cognitive abilities like numerosity, is both innovative and timely. Zebrafish, a valuable model for studying functional asymmetries, offer unique insights into how brain lateralisation influences decision-making processes, which she has demonstrated in her work. Her findings on the distinct number representation systems in newborn guppies and the perceptual strategies of bumblebees further solidify her reputation in the field.

Her expertise in numerosity, brain asymmetry, and the decision-making processes of zebrafish, alongside her advanced imaging techniques learned through collaborations, will provide the symposium with cutting-edge insights into how lateralisation shapes cognitive functions in vertebrates. Dr Miletto-Petrazzini's work is of critical relevance to those studying brain lateralisation, numerosity, animal cognition, and the evolution of cognitive systems, making her an ideal candidate to speak at this symposium.

SYMPOSIUM 02

New approaches to understanding and restoring impaired sensory and motor functions caused by spinal cord injuries: the need for multidisciplinary therapies to combat highly complex diseases

Spinal cord injuries pose significant challenges to motor and sensory systems, underscoring the need to understand the mechanisms driving motor control and how they can be harnessed or restored through cutting-edge approaches. Furthermore, exploring the physiological basis of aberrant neuronal activity in the somatosensory system is crucial, as it contributes to secondary pathologies such as neuropathic pain. This session delves into the anatomical substrates and functional principles of motor and sensory circuits disrupted by spinal cord injuries, presenting innovative strategies for their restoration.

Renowned experts will present cutting-edge techniques to analyze neural circuits and their roles in movement and sensory integration. The symposium will highlight methods such as neuromodulation, biomaterials, optical control, electrophysiology, and viral tracing to explore how brain-spinal pathways coordinate complex functions, emphasizing the integration of these advanced approaches to uncover the mechanisms driving movement generation and sensory processing.

A key emphasis will be placed on translational applications, including the development of therapeutic strategies to repair disrupted motor circuits. Topics will range from fundamental insights into neural circuit function to clinical approaches for promoting motor recovery and restoring function after spinal cord injuries, and the rebalance of the alterations in the somatosensory cortex. This multidisciplinary framework bridges the gap between basic neuroscience and applied therapeutics.

The Symposium is designed to appeal a diverse neuroscience audience by addressing both physiological and pathological states. It aligns with the broader themes of the Spanish neuroscience meeting, emphasizing inclusivity and the value of collaboration across disciplines, career stages, and countries. The speakers' diverse expertise—spanning from molecular neuroscience to system-level analyses—ensures a comprehensive and enriching discussion, fostering interdisciplinary dialogue.
This Symposium will inspire new ideas and collaborations, promising a significant contribution in understanding and restoring motor function.

SPEAKER #1

MARÍA CONCEPCIÓN SERRANO LÓPEZ-TERRADAS

Instituto de Ciencia de Materiales de Madrid, CSIC

- Recent publications of the speaker related to the topic of the symposium:

1. Martínez-Ramírez J, Toldos-Torres M, Benayas E, Villar-Gómez N, Fernández-Méndez L, espinosa FM, García R, Veintemillas-Verdaguer S, Morales MP, Serrano MC*. Hybrid hydrogels support neural cell culture development under magnetic actuation at high frequency. Acta Biomaterialia 2024, 176, 156-172.

2. Girão A*, Serrano MC*, Completo A, Marques P*. Is graphene shortening the path toward spinal cord regeneration? ACS Nano 2022, 16, 13430-13467. Selected as “Featured Content: Recent highlights from the journal” for several weeks.

3. Domínguez-Bajo A, Rosa JM, González-Mayorga A, Rodilla BL, Arché-Núñez A, Benayas E, Ocón P, Pérez L, Camarero J, Miranda R, González MT, Aguilar J, López-Dolado E, Serrano MC*. Nanostructured gold electrodes promote neural maturation and network connectivity. Biomaterials 2021, 279, 121186.

- Relevance for the symposium:

Biomaterials research has widely increased in the last decades, appearing as a solid and versatile platform for ideally engineering any damaged and/or diseased tissue including the nervous system. Particularly, recent advances for neural tissue engineering are providing promising results with a wide plethora of biomaterials including nanomaterials, with and without the incorporation of cell therapy strategies. In our laboratory, we have been exploring biomaterials for neural therapeutics at the injured spinal cord for over 10 years, including graphene derivatives, natural hydrogels and iron oxide nanoparticles. All these biomaterials have already brought insights of neural colonization and growth in rat spinal cord injury models of cervical hemisection and thoracic transection, thus opening a new path in the search of central nervous system therapeutics.

SPEAKER #2

Carmelo Bellardita, PhD

Associated professor, group leader

Neural Circuitries and Motor Repair Laboratory

Department of Neuroscience

Copenhagen University.

- Recent publications of the speaker related to the topic of the symposium:

1-Marcantoni M, Fuchs A, Löw P, Barstch D, Kiehn O and Bellardita C. “Early delivery and prolonged treatment with nimodipine prevent spasticity after spinal cord injury. Science Translational Medicine (2020).

2-H. Wu, C. Petitpré, P. Fontanet, A. Sharma, BELLARDITA C., Y. Wang,1 J. A. Heimel, K. Cheung, S. Wanderoy, Y. Xuan, K.Meletis, J. Ruas, O. Kiehn, S. Hadjab, F. Lallemend, Distinct subtypes of proprioceptive dorsal root ganglion neurons regulate adaptive proprioception in mice. Nature Comunication (2021)

3-Y. Yan, I. Vargas, L. Xu, N. Antolin, G. Kong, I. Palmisano, Y. Yi, J. Chadwick, F. Mueller, A. Bull, C. Lo Celso, G. Primiano, S. Servidei, S. BELLARDITA C.*.Digiovanni*. Macrophages excite muscle spindles with glutamate to bolster locomotion. Nature (2024).

Relevance for the symposium:

Spinal cord injury research is rapidly evolving, aiming to restore movement and function through innovative approaches such as neuromodulation, sensory integration, and cellular therapies. My research focuses on key mechanisms in spasticity prevention, sensory-motor coupling for rehabilitation, and neuro-immune interactions, providing fundamental insights with strong translational potential that align seamlessly with the symposium’s themes.

SPEAKER #3

Kajana Satkunendrarajah, Ph.D.

Associate Professor

Department of Neurosurgery

Associate Director of Neuroscience Research Center

Medical College of Wisconsin

Recent publications of the speaker related to the topic of the symposium:

1. **Karadimas SK, **Satkunendrarajah K, Laliberte AM, Ringuette D, Weisspapir I, Li L, Gosgnach S, Fehlings MG. Sensory cortical control of movement. Nat Neurosci. 2020 Jan;23(1):75-84. PubMed PMID: 31740813. (**Equally contributing authors)

2. Satkunendrarajah K, Karadimas SK, Laliberte AM, Montandon G, Fehlings MG. Cervical excitatory neurons sustain breathing after spinal cord injury. Nature. 2018 Oct;562(7727):419-422. PubMed PMID: 30305735.

3. Sweetman H, Rahman M, Vedantam A, Satkunendrarajah K. Subclinical respiratory dysfunction and impaired ventilatory adaptation in degenerative cervical myelopathy. Experimental Neurology. 2023 Oct 29;371:114600. PubMed PMID: 37907124

- Relevance for the symposium:

This research is centered on the neural mechanisms underlying the recovery of motor functions following traumatic spinal cord injury (SCI). It focuses on key areas, from understanding fundamental circuit functions to developing therapeutics for neurological conditions. The key focus areas include 1) Delineating the neuronal circuits involved in movement and breathing; 2) Plasticity of neural circuits following traumatic and non-traumatic spinal cord injury; 3) developing treatments for the restoration of function following traumatic and non-traumatic spinal cord injury.Traditionally, the primary motor cortex has been considered the main control center for movement. However, our recent findings reveal a significant role for the primary somatosensory cortex (SI) in modulating locomotor function, even in the absence of direct input from the motor cortex. These studies use viral vector systems to selectively target this pathway and observe its effects on spinal circuits in preclinical SCI models.Our findings are not only revealing fundamental insights into motor control but will pave the way for targeted interventions that could restore locomotion in individuals with SCI.

SPEAKER #4

Juan Aguilar. PhD.

Experimental Neurophysiology Group.

Hospital Nacional de Parapléjicos. SESCAM

Instituto de Investigación Sanitaria de Castilla-La Mancha. IDISCAM.

Recent publications of the speaker related to the topic of the symposium:

1.-Alonso-Calviño E, Fernández-López E, Zaforas M, Rosa JM, Aguilar J*. (2023). Increased excitability and reduced GABAergic levels in somatosensory cortex under chronic spinal cord injury. Exp Neurol 369, 114504. doi: 10.1016/j.expneurol.2023.114504

2.-Zaforas M, Rosa JM, Alonso-Calviño E, Fernández-López E, Miguel-Quesada C, Oliviero A, Aguilar J*. (2021). Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury. Journal of Physiology 599(20): 4643-4669. https://doi.org/10.1113/JP281901

3.-Fernández-López E, Alonso-Calviño E, Humanes-Varela D, Foffani G, Aguilar J*. (2019). Slow-wave activity homeostasis in the somatosensory cortex after spinal cord injury. Experimental Neurology. 322:113035. doi: 10.1016/j.expneurol.2019.113035.

- Relevance for the symposium:

Beyond paralysis, a spinal cord injury produces direct and massive damage to the somatosensory system, leading to secondary pathologies such as neuropathic pain and phantom sensation. Nowadays, there is no cure or effective treatment for these associated pathologies, which means a dramatic reduction in the life quality of patients. Although both phenomena have been related to aberrant neuronal activity in different structures of the somatosensory system, the origin and mechanism of the alterations are still unknown.

Our research focuses on characterizing the neuronal bases underlying aberrant activity associated with neuropathic pain and phantom sensation in supraspinal structures of the primary somatosensory system.

To do that, we employ a combination of techniques, including in vivo electrophysiology, live calcium imaging of inhibitory cell populations, and ex vivo patch-clamp recordings spinal cord injured animals. These approaches help us explore the cellular mechanisms driving cortical changes in this context.

Our main results indicate that a spinal cord injury produces increased excitability in the deprived somatosensory cortex, which is observed during spontaneous activity and evoked responses. Moreover, the deafferented cortex shows a reduction on GABAergic activity affecting selectively infragranular layers in chronic conditions. Here we provide a new physiological marker of cortical reorganization and identify neuronal targets to treat aberrant activity in the somatosensory system after a spinal cord injury.

SYMPOSIUM 03

NEURON-GLIA METABOLIC COOPERATION: VITAL FOR ORGANISMAL HOMEOSTASIS AND HEALTH

Major advances have occurred in the understanding of the cellular and molecular determinants of brain energy metabolism highlighting a novel framework of well-regulated and dynamic metabolic dialogues between different cell types. These comprise neuron-astrocyte coupling in the grey matter, and oligodendrocyte-axonal metabolic coupling in the white matter, the mechanisms of which are only beginning to be deciphered. The current conceptual understanding proposes that neuron-astrocyte and axon-oligodendrocyte partnerships function as distinct metabolic units, collaborating intricately to sustain brain function and promote the overall well-being of the organism.

Thus, the metabolism of adjacent neurons and astrocytes/oligodendrocytes is mutually dependent, such as singular functional units committed to secure neurotransmission and neuroprotection. Moreover, reciprocal crosstalk between metabolism and neurotransmission entails metabolites operating beyond mere energy fuels and serving as signals that modulate the function of neurotransmitter receptors and transporters. In this symposium proposal, we aim to update on the specific molecular mechanisms that regulate the metabolic cooperation between both units and how these mechanisms determine synaptic activity and plasticity, as well as axonal integrity. The speakers have complementary expertise, both in methodological approaches and scientific focus, in the study of these metabolic units. The symposium would be an excellent opportunity to exchange novel concepts and show the similarities and differences in metabolic units between grey and white matter. We will first discuss how astrocytic metabolism, particularly the mitochondrial respiratory chain organization, transforms energy metabolism into signals (e.g., ROS) aimed to sustain neuronal function and behavior in the mouse (Bolaños). We will then address how the neuron-glia metabolic interactions operate in Drosophila and how they are adapted to suboptimal conditions; a unique opportunity to establish similarities and differences between species (Schirmeier). Next, we will update how astrocytic metabolism, in particular glycolysis, sustains glutamatergic neurotransmission by using a glycolytic derivative, serine, as a signal that directly modulates NMDA receptors in vivo, both in health and disease, in particular Alzheimers' disease (Bonvento). Finally, we will learn that white matter development and integrity, rather than relying mainly on oligodendroglial glucose catabolism, depends on the capacity of oligodendroglia to import monocarboxylates derived, at least in part, from astrocytes and used both as myelin lipid precursors and axonal energy support, and how this may be altered in multiple sclerosis (Tepavcevic).

We believe that this symposium proposal will be of high interest to a modern Neuroscience community that is aware that only by elucidating the fine regulation of brain metabolism, we will be able to better understand the mechanisms regulating brain function and why this fails.

CHAIR 1

Juan P. Bolanos (co-organizer and Speaker) University of Salamanca (USAL) (Year of PhD: 1992) (https://ibfg.usal-csic.es/juan-pedro-bolanos.html)

- Weak neuronal glycolysis sustains cognition and organismal fitness.
  Jimenez-Blasco D, Agulla J, Lapresa R, Garcia-Macia M, Bobo-Jimenez V, Garcia-Rodriguez D, Manjarres-Raza I, Fernandez E, Jeanson Y, Khoury S, Portais JC, Padro D, Ramos-Cabrer P, Carmeliet P, Almeida A, Bolaños JP.
  Nat Metab. 2024 Jul;6(7):1253-1267. doi: 10.1038/s42255-024-01049-0.
- Fatty acid oxidation organizes mitochondrial supercomplexes to sustain astrocytic ROS and cognition.
  Morant-Ferrando B, Jimenez-Blasco D, Alonso-Batan P, Agulla J, Lapresa R, Garcia-Rodriguez D, Yunta-Sanchez S, Lopez-Fabuel I, Fernandez E, Carmeliet P, Almeida A, Garcia-Macia M, Bolaños JP.
  Nat Metab. 2023 Aug;5(8):1290-1302. doi: 10.1038/s42255-023-00835-6.
- Aberrant upregulation of the glycolytic enzyme PFKFB3 in CLN7 neuronal ceroid lipofuscinosis.
  Lopez-Fabuel I, Garcia-Macia M, Buondelmonte C, Burmistrova O, Bonora N, Alonso-Batan P, Morant-Ferrando B, Vicente-Gutierrez C, Jimenez-Blasco D, Quintana-Cabrera R, Fernandez E, Llop J, Ramos-Cabrer P, Sharaireh A, Guevara-Ferrer M, Fitzpatrick L, Thompton CD, McKay TR, Storch S, Medina DL, Mole SE, Fedichev PO, Almeida A, Bolaños JP.
  Nat Commun. 2022 Jan 27;13(1):536. doi: 10.1038/s41467-022-28191-1.

CHAIR 2

Angeles Almeida (co-organizer) Spanish National Research Council (CSIC) (Year of PhD: 1993) (https://ibfg.usal-csic.es/angeles-almeida.html)

- Cross-talk between energy and redox metabolism in astrocyte-neuron functional cooperation.
  Almeida A, Jimenez-Blasco D, Bolaños JP.
  Essays Biochem. 2023 Mar 3;67(1):17-26. doi: 10.1042/EBC20220075.
- APC/C-Cdh1-targeted substrates as potential therapies for Alzheimer's disease.
  Lapresa R, Agulla J, Bolaños JP, Almeida A.
  Front Pharmacol. 2022 Dec 14;13:1086540. doi: 10.3389/fphar.2022.1086540. eCollection 2022.
- Nuclear WRAP53 promotes neuronal survival and functional recovery after stroke.
  Sánchez-Morán I, Rodríguez C, Lapresa R, Agulla J, Sobrino T, Castillo J, Bolaños JP, Almeida A.
  Sci Adv. 2020 Oct 7;6(41):eabc5702. doi: 10.1126/sciadv.abc5702.

SPEAKER 1

Stefanie Schirmeier (Speaker) Technische Universität Dresden (TU Dresden) (Year of PhD: 2010) (https://www.nwg-info.de/de/council/elections/2023/schirmeier)

Glycolytically impaired Drosophila glial cells fuel neural metabolism via β-oxidation.

McMullen E, Hertenstein H, Strassburger K, Deharde L, Brankatschk M, Schirmeier S.

Nat Commun. 2023 May 24;14(1):2996. doi: 10.1038/s41467-023-38813-x.

McMullen E, Hertenstein H, Müller S, Schirmeier S (2022) Genetically Encoded Sensors to Study Metabolism in Drosophila. In: Drosophila: Methods and Protocols (Dahmann C, ed), pp 401–414. New York, NY: Springer US. Available at: https://doi.org/10.1007/978-1-0716-2541-5_21.

Hertenstein H, McMullen E, Weiler A, Volkenhoff A, Becker HM, Schirmeier S (2021) Starvation-induced regulation of carbohydrate transport at the blood–brain barrier is TGF-β-signaling dependent (VijayRaghavan K, ed.)

Elife 10:e62503 Available at: https://doi.org/10.7554/eLife.62503.

SPEAKER 2

Gilles Bonvento (Speaker) Institut des Neurosciences Paris-Saclay (NeuroPSI) (Year of PhD: 1991)

(https://neuropsi.cnrs.fr/en/departments/cnn/group-leader-gilles-bonvento/)

- Glycolysis-derived L-serine levels versus PHGDH expression in Alzheimer's disease.
  Bonvento G, Oliet SHR, Panatier A.
  Cell Metab. 2022 May 3;34(5):654-655. doi: 10.1016/j.cmet.2022.04.002.
- Astrocyte-neuron metabolic cooperation shapes brain activity.
  Bonvento G, Bolaños JP.
  Cell Metab. 2021 Aug 3;33(8):1546-1564. doi: 10.1016/j.cmet.2021.07.006.
- Impairment of glycolysis-derived l-serine production in astrocytes contributes to cognitive deficits in alzheimer's disease
  Le Douce J, Maugard M, Veran J, Matos M, Jégo P, Vigneron PA, Faivre E, Toussay X, Vandenberghe M, Balbastre Y, Piquet J, Guiot E, Tran NT, Taverna M, Marinesco S, Koyanagi A, Furuya S, Gaudin-Guérif M, Goutal S, Ghettas A, Pruvost A, Bemelmans AP, Gaillard MC, Cambon K, Stimmer L, Sazdovitch V, Duyckaerts C, Knott G, Hérard AS, Delzescaux T, Hantraye P, Brouillet E, Cauli B, Oliet SHR, Panatier A, Bonvento G.
  Cell Metab. 2020 Mar 3;31(3):503-517.e8. doi: 10.1016/j.cmet.2020.02.004.

SPEAKER 3

Vanja Tepavcevic (Speaker) University of Valencia (UV) (Year of PhD: 2006)

(https://www.uv.es/uvweb/instituto-universitario-cavanilles-biodiversidad-biologia-evolutiva/es/investigacion/grupos-investigacion/neurobiologia-comparada/directorio-1285894452524.html)

- Oligodendrocyte progenitor cell recruitment and remyelination in multiple sclerosis: the more, the merrier?
  Tepavčević V, Lubetzki C.
  Brain. 2022 Dec 19;145(12):4178-4192. doi: 10.1093/brain/awac307.
- Genetically modified macrophages accelerate myelin repair.
  Aigrot MS, Barthelemy C, Moyon S, Dufayet-Chaffaud G, Izagirre-Urizar L, Gillet-Legrand B, Tada S, Bayón-Cordero L, Chara JC, Matute C, Cartier N, Lubetzki C, Tepavčević V.
  EMBO Mol Med. 2022 Aug 8;14(8):e14759. doi: 10.15252/emmm.202114759.
- Oligodendroglial Energy Metabolism and (re)Myelination.
  Tepavčević V.
  Life (Basel). 2021 Mar 13;11(3):238. doi: 10.3390/life11030238.

SYMPOSIUM 04

Understanding Habenulae function in Emotional Behavior

Recently, the habenula has emerged as a critical brain region involved in emotional processing and motivational regulation. Structurally, the habenula comprises two distinct divisions, the medial and lateral (MHb and LHb, respectively), which differ significantly in genetic identity and connectivity. Despite these distinctions, both divisions show notable overlap in their functional roles in emotional behavior. This symposium proposes an in-depth examination of the circuits and receptor mechanisms connecting the medial and lateral habenula in the contexts of learned and innate fear, as well as drug-related behaviors.

Susanna Molas will present data linking the MHb projection to the interpeduncular nucleus and its downstream structures with the attenuation of innate fear.

Ines Ibañez-Tallon will show experiments demonstrating that cholinergic receptors and the GPR151 at the MHb control motivational value and could be a new target to treat mood disorders.

Christophe Proulx will report experiments testing the hypothesis that VTA-projecting LHb neurons encode aversive signals required in associative learning to promote escape behavior

Edgar Soria-Gomez will present results analyzing how cannabinoid receptor type-1 signaling at different MHb cell types interacts with peripheral stress signals to define emotional behavior .

In summary, the symposium aims to elucidate how the habenula contributes to the encoding of motivation and emotion across different behavioral states with the ultimate goal of defininning molecular targets that modulate these processes in physiology and pathology.

Publications:

Molas 2024, Nat Commun: 10.1038/s41467-024-47255-y
Molas 2024, Res Sq:10.21203/rs.3.rs-4661779/v1
Molas 2017, Nat Neurosci: 10.1038/nn.4607

Ables 2023, Pharmacol Res: 10.1016/j.phrs.2023.106734
Beatriz Antolin-Fontes 2020, PNAS: 10.1073/pnas.1916132117
Ables 2017, PNAS: 10.1073/pnas.1717506114

Hernandez Silva 2024, J Neurosci: 10.1523/JNEUROSCI.2082-23.2024
Martianova 2023, iScience 10.1016/j.isci.2023.108328
Proulx 2018, PNAS: 10.1073/pnas.1801837115

Soria-Gomez 2021, Neuron: 10.1016/j.neuron.2021.03.007
Oliveira da Cruz 2020, Cell Rep: 10.1016/j.celrep.2020.108046
Soria-Gomez 2015, Neuron: 10.1016/j.neuron.2015.08.035

SYMPOSIUM 05

Excitatory and inhibitory mechanisms mediating dysfunction of neural circuits in neurodegeneration

Recent advances on cell-specific/single-cell profiling and modulation technologies have allowed detailed characterization of specific neural populations during physiological and pathological conditions. In neurodegenerative diseases, early altered activity of brain circuits is associated with transcriptional and pathophysiological changes affecting specific cell types and their interactions, including excitatory and inhibitory neurons. In Alzheimer's disease (AD), excitatory neurons are the main degenerating population, although emerging evidence indicate that early dysfunction of GABAergic interneurons mediate excitatory/inhibitory (E/I) imbalance, leading to hyperexcitability and memory loss. In this symposium, we will present recent findings on the cellular and molecular mechanisms mediating E/I dysfunction of neural circuits in neurodegenerative diseases, including AD.

The four speakers represent diverse expertise and career stages, from early career researchers to Associate and Full Professors.

SPEAKER 1

Dr. Verret will present recent data on the underlying mechanisms mediating dysfunction of hippocampal activity during social memory deficits in AD transgenic (Tg) mice (Cattaud et al. Neurobiol.Aging (2018), PMID:30273829; Rey et al. iScience (2022), PMID:35243253; Szabo et al. Neurobiol.Aging (2023), PMID:36634385).

SPEAKER 2

Dr. van Kesteren will discuss his current research on molecular adaptions of excitatory and inhibitory neurons associated with changes in excitability (Hijazi et al. Mol. Psychiatry (2020), PMID:31431685; Hijazi S, et al. iScience (2020), PMID:32593000; van Heusden et al. Alzheimers.Res.Ther. (2023), PMID:3760839).

SPEAKER 3

Dr. Plass will discuss her ongoing research on gene regulatory networks of AD at single-cell resolution (Plass et al. Science (2018), PMID:29674432; Fernández-Moya et al. Transcription (2023), PMID:38229529; Gutiérrez-Franco et al. Commun.Biol. (2023), PMID:37188816).

SPEAKER 4

Dr. Parra-Damas will present recent unpublished data on the differential impact and interaction of Aβ and tau pathologies on excitatory and inhibitory hippocampal neurons of APP/Tau Tg mice (Parra-Damas et al. Biol.Psychiatry (2017), PMID:27587263; Saura et al. Sem.Cell.Dev.Biol. (2023), PMID:35623983; Capilla et al. bioRxiv (2023), DOI:10.1101/2023.04.12.536603).

SYMPOSIUM 06

Cell fate in human neurodevelopment and disease

In this symposium, we bring together complementary expertise in the neurodevelopment field to tackle specific questions about how the human brain is built, with a focus on cell fate in development, evolution and disease. Sara Bizzotto will talk about somatic mosaicism in the human brain. She will discuss how somatic variants detected in human tissues provide a tool to study cell lineages, and explain the potential and limitations of such studies. Also, she will talk about mosaic developmental brain disorders and show how cell-type-informed genotyping of somatic variants provides new insights into affected lineages and associated transcriptional programs. Ana Uzquiano will discuss the potential of reproducible 3D in vitro models of the human brain to unravel human-specific features of cortical development, including the identification of novel regulators of cellular diversification and the delineation of lineage relations of human cortical cells. Finally, she will discuss how human brain organoids can be leveraged to shed light onto the biology of complex human neurodevelopmental diseases. Gabriel Santpere will be focus on genetic risk mapping in cell types during neurodevelopment with a special focus on the different types of neural progenitors, including brain organizing centers. He will discuss how his lab aims to identify the multiple critical phases of transcription factors involved in neuropsychiatric diseases and cortical malformations by reconstructing cell-type-specific regulatory networks, in humans, both in vitro and in vivo. Finally, Hsiu-Chuan Lin will discuss the potential of leveraging 2D and 3D human iPSC-derived neural model systems to understand cell fate in human neural development. This includes work on 1) Lineage recording in human cerebral organoids, 2) Single-cell multiomic profiling of human 'midbrain organoid' development, and 3) Human neuron subtype programming through combinatorial patterning with scRNA-seq readouts.

Publications DOI:

https://doi.org/10.1101/2024.10.01.615793

10.1126/science.abe1544

https://doi.org/10.1038/s41583-022-00572-x

10.1093/hmg/ddad107

10.1016/j.cell.2022.09.010

10.1038/s41586-021-04358-6

10.1101/2024.06.14.598925

10.1126/science.adf3786

10.1126/science.aat7615

10.1038/s41592-021-01344-8

https://doi.org/10.1101/2023.12.12.571318.

https://doi.org/10.1101/2023.10.05.561097.

SYMPOSIUM 07

Inhibitory circuits supporting network function

MAIN TOPIC

From Ramon y Cajal's histological descriptions to modern genetic studies, neuroscience has identified a rich variety of neuronal types. While principal cells in the cortex are relatively uniform, recent advances in single-cell sequencing have revealed over twenty-five distinct types of inhibitory interneurons in the cortex alone—potentially over 100, depending on the dataset and clustering methods used. This diversity is even more remarkable, considering that interneurons constitute only 15% of all cortical neurons.

Yet, our understanding of how the diversity of interneurons contributes to network functions remains rudimentary. Most research on this topic has focused on just two interneuron types (parvalbumin and somatostatin, accounting for about 90% of published studies in the last 20 years). This narrow focus overlooks the rich variety of interneurons and their potential roles in shaping circuit dynamics. To truly understand how cognitive functions emerge from the interactions of networks of neurons, it is essential to investigate the full spectrum of cortical interneurons diversity and integrate them into experimental and theoretical frameworks.

The genetic methodological revolution of the last two decades has greatly expanded our experimental possibilities. This immense, untamed diversity of interneurons appears to converge into a relatively small number of major genetic families, enabling researchers to target specific interneuron populations through the expression of a few marker genes. For the first time, it is now feasible to identify and manipulate these families, paving the way to uncover their distinct roles within living brain circuits. In a collaborative effort, researchers from various fields have begun incorporating these new dimensions of neuronal diversity into their experimental designs to develop more mechanistic explanations for their questions. For this symposium, we have invited a diverse group of speakers spanning diverse disciplines and career stages, ranging from development to learning and memory. Their work highlights the importance of embracing interneuronal diversity and demonstrates how this perspective is advancing our understanding of fundamental brain mechanisms and cognition.

OVERVIEW OF THE SYMPOSIUM:

Interneurons constitute a minority (15%) of cortical neurons but embody much of the neuronal genetic diversity. What evolutionary advantage might have driven this disproportionate diversity of interneurons? While the scientific community agrees that this diversity is essential for brain function, our ability to dissect their functional roles has been limited until recent years. In this symposium, we bring together four speakers from diverse disciplines, ranging from development to learning and memory, who exemplify the complexity and significance of integrating interneuronal diversity into the investigation of the neural mechanisms of behavior.

SPEAKER 1

Antonio Fernandez-Ruiz

Department of Neurobiology and Behavior. Cornell University, Ithaca (NY), US.

- Liu C, Todorova R, Tang W, Oliva A, Fernandez-Ruiz A. (2023) Associative and predictive hippocampal codes support memory-guided behaviors. Science 382 (6668), eadi8237
- Harvey RE, Robinson HL, Liu C, Oliva A, Fernandez-Ruiz A. (2023) Hippocampo-cortical circuits for selective memory encoding, routing, and replay. Neuron 111 (13), 2076-2090. e9
- Chang H, Tang W, Wulf A, Nyasulu T, Wolf M, Fernandez-Ruiz A*, Oliva A*. (2024). Sleep micro-structure organizes memory replay. Nature (in press)

The fine-tuned coordination of different neuronal populations in distributed brain circuits supports complex cognitive functions, including assemblies of excitatory and inhibitory interneurons. The main goal of Antonio's research is to understand how distributed neuronal dynamics support this process. He approaches this challenge by developing and applying cutting-edge techniques, such as complex behavioral tasks in rodents and large-scale silicon probe recordings combined with closed-loop optogenetic manipulations. For this symposium, he brings a unique perspective that combines his expertise in circuit physiology with his extensive experience in large-scale recording and manipulation technologies.

SPEAKER 2

Jozsef Csicsvari

Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria.

- Dámaris K Rangel Guerrero, Kira Balueva, Uladzislau Barayeu, Peter Baracskay, Igor Gridchyn, Michele Nardin, Chiara Nina Roth, Peer Wulff, Jozsef Csicsvari. (2024) Hippocampal cholecystokinin-expressing interneurons regulate temporal coding and contextual learning. Neuron, 112(12), 2045-2061
- -Gridchyn I, Schoenenberger P, O'Neill J, Csicsvari J (2020) Assembly-Specific Disruption of Hippocampal Replay Leads to Selective Memory Deficit. Neuron, 106(2):291-300.
- Boccara CN , Nardin M, Stella F, O'Neill J, Csicsvari J. (2019) The entorhinal cognitive map is attracted to goals. Science 363 (6434), 1443-1447.

Jozsef studies how learning is implemented in the brain. In his brilliant career, he has pioneered several methods for accessing the neural activity of behaving animals, which today have become field standards. More recently, his group has been investigating the mnemonic role of neuronal populations and their interactions in brain areas involved in spatial memory processing, including specific subtypes of interneurons. As a leading expert in the field, he provides to this symposium an in-depth exploration of how neuronal circuits in the hippocampus and related brain areas are involved in spatial memory processing, highlighting their role in learning and memory consolidation.

SPEAKER 3

Laura Modol

Deparment of Neuroscience. Hospital del Mar Research Institute, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain.

- Mòdol L, Moissidis M, Selten M, Oozeer F, Marín O. Somatostatin interneurons control the timing of developmental desynchronization in cortical networks. Volume 112, Issue 12p2015-2030.e5June 19, 2024.
- Mòdol L, Bollmann Y, Tressard T, Baude A, Che A, Duan ZRS, Babij R, De Marco García NV, Cossart R. Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex. Volume 105, Issue 1p93-105.e4January 08, 2020.
- Bollmann* Y, Mòdol* L, Tressard T, Vorobyev A, Dard R, Brustlein S, Sims R, Bendifallah I, Leprince E, de Sars V, Ronzitti E, Baude A, Adesnik H, Picardo MA, Platel JC, Emiliani V, Angulo-Garcia D, Cossart R. Prominent in vivo influence of single interneurons in the developing barrel cortex. Nature Neuroscience 26 (9), 1555-1565.

Spontaneous activity is a hallmark of developing circuits, necessary for the formation and wiring of neural networks and for efficient information processing in the adult brain. Using a combination of optical, genetic, and computational tools, Laura's work has been fundamental to understanding the importance of GABAergic interneuron diversity in regulating developmental dynamics and driving the maturation of cortical circuits. Her expertise and scientific contributions will bring a unique developmental perspective to the symposium, complementing the talks focused on the role of interneurons in shaping mature circuit function.

SPEAKER 4

Manuel Valero

Department of Neuroscience. Hospital del Mar Research Institute, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain.

- Valero M*, Zutshi I, Yoon E, Buzsáki G*. Probing subthreshold dynamics of hippocampal neurons by pulsed optogenetics. Science 375 (6580), 570-574. (2022).
- Valero M, Navas-Olive A, Menendez de la Prida L, Buzsáki G. Inhibitory conductance controls place field dynamics in the hippocampus. Cell Reports 40 (8), 111232. (2022).
- Valero M, Viney T, Machold R, Mederos S, Zutshi I, Schuman B, Senzai Y, Rudy B & Buzsáki G. Sleep down state-active ID2/Nkx2.1 interneurons in the neocortex. Nature Neuroscience 24, 401-411. (2021).

The interplay between excitatory and inhibitory activity in cortical networks is believed to underlie the creation of neural representations and memories. Throughout his career, Manu has offered a unique single-cell perspective on this phenomenon, investigating how single-cell computations scale up to network-level functions. In recent years, by combining specific recombinase lines with novel high-resolution optogenetic techniques, he has launched a scientific program focused on understanding the role of different interneuron subtypes in shaping these single-cell computations.

SYMPOSIUM 08

Multiscale approximations to understand microglia dysfunction in Alzheimer's disease: from genes to cells, looking for new therapeutic targets

OVERVIEW: This symposium will address the relevance of microglia states in the Alzheimer's disease pathological course through a multi-scale, multi-species focus. The symposium will present state-of-the-art approximations, including single cell transcriptomics, cell profiling and microstructural neuroimaging in both animal models and human tissue.

Convergent evidences demonstrated that neuroinflammation through microglia reactivity is a key aspect of Alzheimer's disease (AD). While the amyloid cascade hypothesis falls short in explaining various facets of AD, the elevated inflammatory markers since preclinical stages and the discovery of risk genes linked to innate immunity entail microglia as a key player. However, their position in the pathological cascade is incompletely understood, preventing optimal development of diagnostic and prognostic approaches, and the discovery of new therapeutic targets and disease-modifying treatments. Here, we want to engage the audience by offering different perspectives on microglia dynamics in AD, ranging from sub-cellular to whole brain, and from rodent models to humans.

Once the classic dichotomy between resting and active has been overcome, single-cell transcriptomics has emerged as the preferred approach to identify microglia states, linking their heterogeneity to disease trajectories. However, microglia are not alone: they are in constant communication with other cells; by profiling multiple cell types simultaneously, it is possible to uncover novel mechanisms by which inter- and intra-cellular signals integrate to achieve homeostasis in the neuroglial unit, and uncover mechanisms of non cell-autonomous neurotoxicity in AD. Translational studies in humanized AD rodent models and post-mortem human tissue are fundamental to discern patterns relevant to inform new therapeutic routes. Finally, recent advances based on the use of non-invasive imaging gave access to the characterization of microglia morphology in vivo with a systemic approach, complementing the molecular and cellular probes with a global perspective and allowing the unbiased identification of vulnerable/resistant targets. Since AD disproportionately affects women, the sex dimension will be taken into account at all times. Importantly, the symposium will have a strong translational character, since the speakers will present results focusing on animal models of AD lacking microglia, humanized AD animal models, and human AD tissue post-mortem.

The development of the Symposium will be guided by the principles of the code of conduct of the SENC.

SPEAKER 1

Jose Lopez-Atalaya, Instituto de Neurociencias de Alicante, CSIC-UMH

- Bhojwani-Cabrera, A.M., Navarron-Izquierdo, C.M., Marquez-Galera, A., Exposito-Coca, M.A., Laguna-Luque, M.L., Royo, M., Lopez-Lopez, V., Fuster-Fullana, L., Guillot-Fernández, M., Peixoto, D.O., Pozzi, V., Niñerola, S., Barco, A., Sanchez-Mut, J.V., Gruart, A., Jurado, S., Delgado-García, J.M., Lopez-Atalaya, J.P. Loss of RelA causes microglia reprogramming to an interferon-responsive state, leading to neural plasticity deficits and cognitive dysfunction. In preparation.
- Lopez-Atalaya JP, Bhojwani-Cabrera AM. Interferon-responsive microglia (IRM): friend or foe? In press.
- Rueda-Carrasco J, Martin-Bermejo MJ, Pereyra G, Mateo MI, Borroto A, Brosseron F, Kummer MP, Schwartz S, López-Atalaya JP, Alarcon B, Esteve P, Heneka MT, Bovolenta P. SFRP1 modulates astrocyte-to-microglia crosstalk in acute and chronic neuroinflammation. EMBO Rep. 2021 Nov 4;22(11):e51696. doi: 10.15252/embr.202051696.

Dr. Jose P. López-Atalaya's research focuses on the molecular underpinnings of aging and age-related neurodegenerative diseases that cause dementia. To this aim, his group is using an integrated approach that combines single-cell genomics and cell population-based genomics analysis with mouse genetics. Their latest, as-yet unpublished findings reveal the transcription factor RelA/p65 as a master regulator of microglia homeostasis. They have found that loss of RelA/p65 elicits microglia reprograming toward interferon-responsive phenotype that is associated to neural plasticity deficits and cognitive dysfunction. This work offers new insight into how microglial neuroinflammatory response contributes to cognitive impairment in Alzheimer's disease.

SPEAKER 2

Giles E Hardingham, University of Edinburgh

- M Li, K Holt, K Ridley, J Qiu, K Haddow, D Vasoya, X He, J Tulloch, D King, D A Hume, C Pridans, O R Dando, T L Spires-Jones, G E Hardingham. Microglia determine beta-amyloid plaque burden but are non-essential for downstream pathology. bioRxiv, 2024.08. 06.606795
- P Baxter, O Dando, O., E K, X He, S McKay, G E Hardingham*, J Qiu.* Microglial identity and inflammatory responses are controlled by the combined effects of neurons and astrocytes. Cell Reports 34, 108882 (2021).
- Z Jiwaji, S S Tiwari, ..., Giles E Hardingham. Reactive astrocytes acquire neuroprotective as well as deleterious signatures in response to Tau and Aß pathology. Nature communications 13, 135 (2022).

Prof. Hardingham's research focuses on signaling events within and between neurons and glia, and how these interactions shape brain development and homeostasis as well as drive pathological cascades. Latest results from his team offer novel evidences clarify the role of microglia in plaque formation and downstream pathology: they found that microglia promote plaque formation and creation of an Aß fibril-rich zone surrounding the plaque core, but plaque-proximal reactive astrogliosis, synapse loss, and neurite dystrophy are still observed in the absence of microglia. These results are expected to highly engage the audience for an in-depth scientific discussion.

SPEAKER 3

Antonia Gutiérrez Pérez, University of Malaga

- C Muñoz-Castro, M Mejias-Ortega, ... , A Gutierrez*, J Vitorica*. Monocyte-derived cells invade brain parenchyma and amyloid plaques in human Alzheimer's disease hippocampus. Acta Neuropathol Commun. 11(1):31 (2023).
- R March-Diaz, N Lara-Ureña, ... , A Gutierrez, J Vitorica, A Pascual. Hypoxia compromises the mitochondrial metabolism of Alzheimer's disease microglia via HIF1. Nature Aging 1:385 (2021).
- A Gutierrez, J Vitorica. Toward a New Concept of Alzheimer's Disease Models: A Perspective from Neuroinflammation. J Alzheimers Dis. 64:S329-S338 (2018).

Prof. Antonia Gutiérrez's research addresses various aspects of Alzheimer's pathology with a highly translational focus, with key areas of interest including the role of amyloid-β oligomers, microglial reactivity and neuroinflammation, lysosomal dysfunctions and synaptic damage. Importantly, her group has recently highlighted the role of infiltrating monocyte-derived cells in human AD pathology, offering novel insights to inform the development of new treatment routes. In addition, Prof. Gutiérrez's group is expert in the role of other glial cells like astrocytes in the context of neurodegeneration.

SPEAKER 4

Silvia De Santis, Instituto de Neurociencias de Alicante, CSIC-UMH

- R Garcia-Hernandez, A Cerdán Cerdá, A Trouve Carpena, M Drakesmith, K Koller, D K Jones, S Canals, S De Santis. Mapping microglia and astrocyte activation in vivo using diffusion MRI. Science Advances 2022 8(21):eabq2923
- P Martínez-Tazo, A Santos, M K Selim, E Espinós-Soler, S De Santis. Sex matters: The MouseX DW-ALLEN Atlas for mice diffusion-weighted MR imaging. NeuroImage 2024, 292, 120573.
- N Toschi, R Arrais Gisbert, L Passamonti, S Canals, S De Santis. Multi-shell diffusion imaging reveals gender-specific trajectories of early white matter degeneration in normal aging. Neurobiology of aging 2020;86:191-200.

- Relevance for the symposium: Dr. De Santis' research group recently introduced a non-invasive diffusion-weighted magnetic resonance imaging (dw-MRI) method for mapping microglia morphology in the brain. The study demonstrates the method's effectiveness in distinguishing inflammation from neurodegeneration and its potential for application in human clinical research. In addition, the laboratory has developed novel imaging tools with equitable sex representation, specific for performing unbiased research in preclinical degeneration models. The laboratory has recently received the support of the Pasqual Maragall Foundation through a research grant.

SYMPOSIUM 09

Decoding Astrocyte-Neuron dynamics in brain function

The symposium Decoding Astrocyte-Neuron Dynamics in Brain Function addresses the pivotal role astrocytes play alongside neurons in shaping brain activity and complex behaviors. Astrocyte-neuron signaling represents a cutting-edge frontier in neuroscience, offering new insights into brain function that extend beyond traditional neuron-focused research. Astrocytes, a type of glial cell once considered merely supportive, are now recognized as active participants in the brain's signaling processes. They modulate synaptic transmission, regulate blood flow, and play critical roles in neurovascular coupling and metabolic support to neurons. This symposium highlights cutting-edge discoveries by experts in the field revealing how astrocytes contribute also to higher brain functions, such as memory formation, learning, and emotional processing. Therefore, astrocyte-neuron "crosstalk" results essential for understanding the brain as an integrated system rather than as isolated parts.

The brain's computational models are also evolving, with astrocyte inclusion enabling simulations that are more biologically accurate, further emphasizing the importance of astrocytes in neural circuitry. This dual rise in technology and conceptual shift toward a holistic understanding of brain function makes now an ideal moment to intensify focus on astrocyte-neuron dynamics. Bridging this gap in neuroscience could be transformative for both basic research and clinical interventions, ultimately expanding our grasp of cognition, consciousness, and overall mental health.

The advancements in imaging and molecular technologies, such as two-photon microscopy, optogenetics, and high-resolution gene expression profiling allow researchers to visualize and manipulate astrocyte and neuron interactions in real-time and at unprecedented levels of detail, paving the way for discoveries that were previously inaccessible. Considering that neurodegenerative and psychiatric conditions, such as Alzheimer's disease, Parkinson's, depression, and schizophrenia are increasingly linked to glial dysfunction, the symposium Decoding Astrocyte-Neuron dynamics in brain function is particularly relevant and timely to understanding how astrocyte-neuron interactions becomes crucial for developing more effective and targeted treatments.

SPEAKER 1

Dmitri Rusakov, UCL Queen Square Institute of Neurology, University College London. UK.

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SPEAKER 2

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SPEAKER 3

Christian Henneberger, Institute of Cellular Neurosciences, University of Bonn. Germany

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SYMPOSIUM 10

Unraveling Neurodevelopmental Disorders: from mouse models to human brain organoids

Neurodevelopmental disorders (NDDs) exist in various forms that altogether affect millions of people. For a proportion of patients with NDDs, genetic screening enables the identification of a rare mutation that is likely to play a major causal role. However, major barriers remain towards the long-term goal of precision treatments for NDDs based on genetic diagnosis.

Crucial among those barriers are the lack of basic understanding of the underlying cellular, molecular, and neurodevelopmental impact of pathogenic patient mutations.

The analysis of animal models of neurological disease has been instrumental in furthering our understanding of NDDs. However, animal models are limited in revealing some fundamental aspects of development, genetics, pathology, and disease mechanisms that are unique to humans. 3D culture based on pluripotent stem cells enable to recreate key molecular and architectural features of brain tissue thus providing a model system for studying poorly-understood processes of human brain physiology and disease. However, questions remain regarding whether these cell-culture-based models are sufficient.

The aim of this Symposium is to bring together researchers from the fields of stem cell reprogramming and brain organoids as well as experts in developmental biology and neurobiology to jointly discuss: i) the need of increasing our comprehension of molecular mechanism(s) underlying human neurodevelopment; ii) the challenges that exist in understanding the uniquely human aspects of NDDs; and iii) the design of new targeted disease-modifying therapies for NDDs. Advances in neural differentiation of pluripotent stem cells, including 3D brain organoids, and their contribution to the understanding of human brain development and disease, will be also discussed.

In summary, we will debate major outstanding questions regarding how basic science research using cellular and animal models can reveal the impact of NDD-associated mutations, how it can open new avenues towards the treatment of NDDs and advance the overall understanding of the human brain. The proposal is transdisciplinary as it will cover several of the main topics of the SENC Meeting: Developmental Neurobiology; Cognitive and Behavioral Neuroscience; Disorders, Nervous System repair and Healthy Aging and New Methods and Technologies.

The development of the Symposium will be guided by the principles of the code of conduct of the SENC (https://www.senc.es/wp-content/uploads/2024/07/Code-of-Conduct.-ENG.pdf).

Speaker #1:

Silvia Capello, Developmental Neurobiology Research Group, Max Planck Institute of Psychiatry, Munich (Germany).

- Forero A, Pipicelli F, Moser S, ...Di Giaimo, R & Silvia Cappello. Extracellular vesicle-mediated trafficking of molecular cues during human brain development. Cell Reports, 2024; 43, 1, 114775.
- Pipicelli F, Baumann N, ... Jabaudon D & Silvia Cappello. Non-cell autonomous regulation of interneuron specification mediated by extracellular vesicles. Science Advances, 2023, 9(20):eadd8164
- Kyrousi C, O'Neill AC, ... Robertson SP & Silvia Cappello. The extracellular matrix protein LGALS3BP regulates progenitor position and relates to human cortical complexity. Nature Communications, 2021, 12, 6298.

Relevance:

Cellular crosstalk is an essential process during brain development and is influenced by numerous factors, including cell morphology, adhesion, the local extracellular matrix and secreted vesicles. Inspired by mutations associated with neurodevelopmental disorders, our lab focus on understanding the role of extracellular mechanisms essential for the proper development of the human brain. Therefore, we combine 2D and 3D in vitro human models to better understand the molecular and cellular mechanisms involved in progenitor proliferation and fate, migration and maturation of excitatory and inhibitory neurons during human brain development and tackle the causes of neurodevelopmental disorders. In particular, we focus on mutations in genes affecting cell-cell contacts, extracellular matrix and vesicle secretion to study intrinsic and extrinsic mechanisms contributing to brain formation. Our data reveal an important contribution of cell-non-autonomous mechanisms in causing neurodevelopmental disorders.

Speaker #2:

Alysson R. Muotri, Dep. of Pediatrics and Cellular & Molecular Medicine at the University of California (San Diego, California). He will be working and traveling in Europe during August/September 2025.

- Courchesne E, Taluja V, Nazari S, Aamodt CM, Pierce K, Duan K, Stophaeros S, Lopez L, Barnes CC, Troxel J, Campbell K, Wang T, Hoekzema K, Eichler EE, Nani JV, Pontes W, Sanchez SS, Lombardo MV, de Souza JS, Hayashi MAF, Muotri AR. Embryonic origin of two ASD subtypes of social symptom severity: the larger the brain cortical organoid size, the more severe the social symptoms. Mol Autism. 2024 May 25;15(1):22.
- Goldberg G, Coelho L, Mo G, Adang LA, Patne M, Chen Z, Garcia-Bassets I, Mesci P, Muotri AR. TREX1 is required for microglial cholesterol homeostasis and oligodendrocyte terminal differentiation in human neural assembloids. Mol Psychiatry. 2024 Mar;29(3):566-579.
- Papes F, Camargo AP, de Souza JS, Carvalho VMA, Szeto RA, LaMontagne E, Teixeira JR, Avansini SH, Sánchez-Sánchez SM, Nakahara TS, Santo CN, Wu W, Yao H, Araújo BMP, Velho PENF, Haddad GG, Muotri AR. Transcription Factor 4 loss-of-function is associated with deficits in progenitor proliferation and cortical neuron content. Nat Commun. 2022 May 2;13(1):2387.

- Relevance for the symposium:

Muotri´s lab is studying the brain from an evolutionary and developmental perspective, differentiating stem cells to recreate "brain organoids" in the controlled setting of a lab. This work has implications for the generation of human disease models by determining the molecular and cellular mechanisms driving neurological complex disorders, such as autism. It is also creating opportunities for identifying and testing novel therapeutic approaches; the nature of this work reduces the amount of time required for moving new drugs to clinical trials. Gaining a clearer understanding of human brain evolution is crucial for interpreting human genetic variants which lead to disease.

Speaker #3:

Antonella Consiglio; Stem Cells and Neurodegeneration Lab

Bellvitge Biomedical Research Institute (IDIBELL), University of Barcelona (Spain). Co-Chair

- Pons-Espinal M, Blasco-Agell L, Fernandez-Carasa I, Andrés-Benito P, di Domenico A, Richaud-Patin Y, Baruffi V, Marruecos L, Espinosa L, Garrido A, Tolosa E, Edel MJ, Otero MJ, Mosquera JL, Ferrer I, Raya A, Consiglio A. Blocking IL-6 signaling prevents astrocyte-induced neurodegeneration in an iPSC-based model of Parkinson's disease. JCI Insight. 2024 Feb 8;9(3): e163359.
- Tristán-Noguero A, Fernández-Carasa I, Calatayud C, Bermejo-Casadesús C, Pons-Espinal M, Colini Baldeschi A, Campa L, Artigas F, Bortolozzi A, Domingo-Jiménez R, Ibáñez S, Pineda M, Artuch R, Raya Á, García-Cazorla À, Consiglio A. iPSC-based modeling of THD recapitulates disease phenotypes and reveals neuronal malformation. EMBO Mol Med. 2023 Feb 6:e15847
- 3.di Domenico A., Carola G., Calatayud C., et al. Consiglio A.* iPSC-derived astrocytes contribute to non-cell autonomous neurodegeneration in Parkinson's disease. Stem Cell Reports., 2019; 12(2):213-229. IF: 6.537, ISI. Cit: 226.

- Relevance for the symposium:

Research in Consiglio's group aims to develop advanced in vitro human disease models, including 3D brain organoids and iPSC-based disease models, which may have important clinical outcomes, such as development of diagnostic tools and novel therapeutic approaches for neurodegenerative diseases, namely Parkinson's disease (PD). Beyond PD, we are also working on Tyrosine Hydroxylase deficiency (THD), a rare genetic disease which leads to Pediatric Parkinsonism (PP). We are in the process of identification and subsequent validation of therapeutic possibilities which aim to restore a healthy phenotype. Recently, in collaboration with Dr. Dalmau and Dr. Martínez from the Hospital Clinic, Barcelona, we began to pursue a stem cell-based diagnostic approach for detection of autoimmune encephalitis.

Speaker #4:

- Full name and affiliation: Aixa V. Morales, Molecular control of Neurogenesis lab, Instituto Cajal (CSIC), Madrid (Spain). SENC Member since 2009. Co-chair.

- Medina-Menéndez, C.*, Li, L.*, Tirado-Menéndez, P.*, Rodríguez-Martín, P., Melgarejo, E., Díaz, M., Valdés-Bescós, M., López-Sansegundo, R. and Morales, A.V. (AC). (9/9) Early postnatal control of quiescence dynamics in neural stem cells is essential for long-lasting neurogenesis (BioRxiv: https://www.biorxiv.org/content/10.1101/2024.05.03.592315v1).
- Tenorio-Castano J, Gómez ÁS, Coronado M, ..., Morales AV, Nevado J, Lapunzina P. (10/12) (2023) Lamb-Shaffer syndrome: 20 Spanish patients and literature review expands the view of neurodevelopmental disorders caused by SOX5 haploinsufficiency. Clinical Genetics 104(6):637-647
- Li, L.*, Medina, C.*, García-Corzo, L., ..., Morales, A.V (AC). (17/17) (2022) SoxD genes are required for adult neural stem cell activation. Cell Reports 38: 110313; [Cover].

- Relevance for the symposium:

A.V. Morales ´s group is devoted to understanding the molecular mechanisms that control neurogenesis in the developing and adult brain. They are interested in the molecular basis of NDDs such as LAMSHF caused by mutations in SOX5. Her laboratory has generated several mouse models of conditional loss of Sox5 in the CNS, including the CA2 hippocampal region, in which they have identified a role of Sox5 in social memory. Further, in the context of adult neural stem cells generation in the developing hippocampus, her group has generated a conditional mouse line for the CNS in which they are currently exploring the relevance of Sox5 in spatial memory. Her group, in collaboration with the “LAMSHF Spain” patient association and La Paz Hospital geneticists, has recently published the first genetic and clinical analysis of Spanish LAMSHF patients.

SYMPOSIUM 11

Neural Mechanisms for Internal State-Dependent Animal Behavior

Animal behavior is not deterministic, that is, the same sensory input will not always lead to the same behavioral output. Instead, the behavioral response exhibited by an animal is strongly influenced by its internal state, which is determined by complex body-brain interactions that include metabolic and hormonal communication as well as past experiences. A clear example of this is found in food-seeking behavior. An animal doesn't always eat a discovered food source. Instead, its decision depends on various contextual and internal signals, such as its metabolic state, energy cost, and potential threats from predators. Consequently, the animal may prioritize safety or rest over eating if it isn't hungry, too tired, or frightened. Understanding the complexity of how the brain integrates sensory evidence with the internal state to shape behavior is central to this symposium, which aims to showcase cutting-edge research revealing a wide array of neural mechanisms that support these flexible decisions.

In this symposium, each speaker will provide insights into how internal states influence animal behavior through various neural mechanisms. Dr. Petzold will discuss how hypothalamic neural ensembles prioritize behaviors based on social and metabolic needs (Petzold et al., Cell Metabolism 2023). Dr. Stempel will explore the integration of internal and external factors in threat escape decisions (Evans, Stempel et al., Nature 2018). Dr. MacAskill will examine how hunger influences goal-directed actions via a specific neural circuit (Wee et al., Neuron 2024). Finally, Dr. Gutierrez-Castellanos will analyze how female mice modify sociosexual behaviors according to their reproductive states (Gutierrez-Castellanos et al., Neuron 2024). Together, these presentations will illustrate, at multiple scales—from subcellular to neural population levels—the neural mechanisms that enable animals to adapt their actions to changing physiological and environmental conditions.

SPEAKER 1

Dr. Nicolas Gutierrez Castellanos. Postdoctoral fellow at Susana Lima's lab (Champalimaud Foundation, Lisbon, Portugal) and incoming Ramon y Cajal investigator at the University of Valencia.

SPEAKER 2

Dr. Andrew MacAskill, Group leader at University College London (UCL).

SPEAKER 3

Dr. Vanessa Stempel, Group leader at Max Planck Institute for Brain Research, Frankfurt, Germany.

SPEAKER 4

Dr. Anne Petzold. Postdoctoral fellow at Tatiana Korotkova's lab (Center for Physiology, Institute for Systems Physiology & Pathophysiology, Cologne, Germany)

SYMPOSIUM 12

Antibody-mediated mechanisms in autoimmune neurological disorders

This symposium will address the expanding role of neural cell-surface antibodies in neurological diseases. These antibodies, which can target ion channels, receptors, and synaptic proteins, are associated with a wide range of neurological symptoms. Notably, antibodies against the N-methyl-D-aspartate receptor (NMDAR) are prevalent in patients who exhibit prominent psychiatric symptoms, memory deficits and other neurological alterations.

Recent advances in neuroimmunology have focused on understanding the mechanisms underlying antibody-mediated encephalitis through cellular and animal models. Cellular models, utilizing imaging and electrophysiological techniques, have described how autoantibodies interact with cell-surface proteins, disrupting normal function. Studies involving passive transfer of patients' antibodies into animal models have further demonstrated their pathogenicity in vivo, linking molecular and behavioral changes. Current research is now exploring active immunization models, which allow for a more comprehensive understanding of the disorder's immunobiology and facilitate testing of novel therapeutic approaches.

In addition to direct effects on their target receptor or synaptic protein, antibodies can also activate innate immune cell-mediated effector functions. Recent findings suggest that innate immune activation, such as microglia, contributes to antigen loss and neuronal dysfunction. However, the mechanisms through which neural cell-surface antibodies contribute to the diversity of clinical phenotypes remain poorly understood, and systematic profiling of the biophysical properties of patients' antibodies is essential.

Furthermore, patients' antibodies have proven to be a valuable tool for understanding core cognitive processes. Recent studies in autoimmune encephalitis patients demonstrate that antibodies can modulate neuronal circuits involved in working memory, revealing potential insights into broader cognitive functions. This symposium presents a comprehensive review on antibody-mediated mechanisms in immune-mediated neurological disorders from multiple perspectives, making it especially timely given the potential for therapeutic advancements that arise from understanding these mechanisms.

SYMPOSIUM 13

Beyond Shaping Vision: Advances in Circuit Assembly and Plasticity

Humans are predominantly visual creatures, relying extensively on sight to navigate and interpret the world. As part of the central nervous system, the visual system has emerged as one of the most informative models for studying neural circuits' development, assembly, and function. Sensory information is captured by the retina, where it is pre-processed and then relayed through sequential pathways: from retinal ganglion cells to the optic chiasm, thalamus, and finally to the primary visual cortex, where higher-order processing unfolds. A crucial component, and possibly an evolutionary advantage, is the ability to adapt sensory processing to changing environmental conditions.

This symposium brings together leading experts to provide a comprehensive overview of current research on the visual system's functionality and plasticity. It presents recent views of how the building of the visual system and visual responses depend on complex circuital, cellular, and functional responses to environmental stimuli. It highlights important implications for our understanding and intervening in neurodevelopmental disorders. Eloísa Herrera will unveil recent advances in understanding the impact of retinothalamic rewiring on cortical network formation. Robert Hindges will present how early visual experience induces cellular and functional plasticity in the retina and changes behaviour. Marta Nieto will examine how inhibitory interneurons influence plasticity in the visual cortex and contribute to building up visual-auditory crossmodal circuits, as well as identify possibilities for repair. Lastly, Tommaso Pizzorusso will explore how experience-dependent plasticity shapes the visual cortex circuitry. The speakers will showcase cutting-edge technologies and the latest discoveries using a variety of model systems, aiming to inspire new ideas and foster future collaborations.

SPEAKER 1

Eloisa Herrera

- Negueruela S, Morenilla-Palao C, Herrera M, Coca Y, Florez-Paz D, López-Cascales MT, Gomis A, Herrera E*. (2024). Proper Frequency of Perinatal Retinal Waves Is Essential for the Precise Wiring of Visual Axons in Nonimage-Forming Nuclei. J Neurosci. 2024 Oct 2;44(40):e1408232024. doi: 10.1523/JNEUROSCI.1408-23.2024.
- Fernández-Nogales M, López-Cascales MT, Murcia-Belmonte V, Escalante A, Fernández-Albert J, Muñoz-Viana R, Barco, A & Herrera E* (2022). Multiomic Analysis of Neurons with Divergent Projection Patterns Identifies Novel Regulators of Axon Pathfinding. Advanced Science. doi: 10.1002/advs.202200615
- Morenilla-Palao C, López-Cascales MT, López-Atalaya JP, Baeza D, Calvo L, Barco A, Herrera E* (2020). A Zic2-regulated switch in a non-canonical Wnt/ß-catenin pathway is essential for the formation of bilateral circuits.
Science Advances 6(46): eaaz8797. DOI: 10.1126/sciadv.aaz8797

SPEAKER 2

Robert Hindges

- Antinucci, P., Suleyman, O., Monfries, C. & Hindges, R. (2016). Neural Mechanisms Generating Orientation Selectivity in the Retina. Current Biology 26: 1802-1815. DOI: 10.1016/j.cub.2016.05.035, PMID: 27374343
- Cheung A., Schachermayer G., Biehler A., Wallis A., Missaire M. & Hindges R. (2022) Teneurin paralogues are able to localise synaptic sites driven by the intracellular domain and have the potential to form cis-heterodimers, Front. Neurosci. 16:915149 doi: 10.3389/fnins.2022.915149 PMID: 36408396
- Gogou C, Beugelink JW, Frias CP, Kresik L, Jaroszynska N, Drescher U, Janssen BJC, Hindges R & Meijer DH. (2024). Alternative splicing controls teneurin-3 compact dimer formation for neuronal recognition. Nat Commun. 15:3648. doi: 10.1038/s41467-024-47763-x. PMID: 38684645; PMCID: PMC11058771.

SPEAKER 3

Marta Nieto

- Bragg-Gonzalo L, Aguilera A, C. González-Arias C, De León Reyes N. S., Sánchez-Cruz A, Carballeira P, Leroy F, Perea G, Nieto M*. Early Cortical GABAergic interneurons determine the projection patterns of L4 excitatory neurons. $ equal first author. Sci Adv. 2024. 10 May 2024. Vol 10, Issue 19. DOI: 10.1126/sciadv.adj99
- Martín-Fernández F, Bermejo-Santos A, Bragg-Gonzalo L, Briz CG, Serrano-Saiz E, Nieto M*. Role of Nrp1 in controlling cortical inter-hemispheric circuits. Elife. 2022 Mar 1;11:e69776. doi: 10.7554/eLife.69776
- De León Reyes NS, Mederos S, Varela I, Weiss LA, Perea G, Galazo MJ, Nieto M*. Transient callosal projections of L4 neurons are eliminated for the acquisition of local connectivity. Nat Commun. (2019) Oct 7;10(1):4549. doi: 10.1038/s41467-019-12495-w

SPEAKER 4

Tommaso Pizzorusso

- Lupori L, Totaro V, Cornuti S, Ciampi L, Carrara F, Grilli E, Viglione A, Tozzi F, Putignano E, Mazziotti R, Amato G, Gennaro C, Tognini P, Pizzorusso T*. A comprehensive atlas of perineuronal net distribution and colocalization with parvalbumin in the adult mouse brain. Cell Rep. 2023 Jul 25;42(7):112788. doi: 10.1016/j.celrep.2023.112788. Epub 2023 Jul 11. PMID: 37436896.
- Napoli D, Lupori L, Mazziotti R, Sagona G, Bagnoli S, Samad M, Sacramento EK, Kirkpartick J, Putignano E, Chen S, Terzibasi Tozzini E, Tognini P, Baldi P, Kwok JC, Cellerino A, Pizzorusso T*. MiR-29 coordinates age-dependent plasticity brakes in the adult visual cortex. EMBO Rep. 2020 Nov 5;21(11):e50431. doi: 10.15252/embr.202050431. Epub 2020 Oct 7. PMID: 33026181; PMCID: PMC7645255.
- Lupori L, Cornuti S, Mazziotti R, Borghi E, Ottaviano E, Cas MD, Sagona G, Pizzorusso T*, Tognini P*. The gut microbiota of environmentally enriched mice regulates visual cortical plasticity. Cell Rep. 2022 Jan 11;38(2):110212. doi: 10.1016/j.celrep.2021.110212. PMID: 35021093.

SYMPOSIUM 14

Gliotransmission and Metabolic Interplay

Astrocyte control of brain physiology is one of the hot topics in neuroscience. Although historically considered passive cells, accumulating evidence has revealed that astrocytes actively participate in regulating brain activity and behavior. They do this through metabolic processes but also by releasing neuroactive substances, termed gliotransmitters, that interact with neurons to regulate their activity. In this symposium we will review the most recent findings regarding how astrocytes regulate neuronal activity and synaptic plasticity in different brain circuits to impact animal behavior. The selected speakers are recognized experts in the field, each offering insights into different neuronal networks and expertise ranging from synaptic physiology to behavior.

Ana Covelo will describe how astrocytes participate in the brain reward system by regulating synaptic plasticity and dopamine effects in the Nucleus accumbens. Paola Bezzi will show the involvement of astrocytes in dopamine homeostasis in the prefrontal cortex and its impact in animal behavior. Cristina García-Cáceres will review how hypothalamic astrocytes control systemic metabolism. Finally, Giovanni Marsicano will explain how the cross-talk between astrocyte energy metabolism and gliotransmission impacts synaptic and cognitive processes.

Therefore, this symposium will provide an opportunity for neuroscientists to gain deeper insights into the most up-to-date findings and techniques related to astrocyte-neurons interactions.

SPEAKER 1

Ana Covelo:

- Nanclares et al. Acta Neuropathologica 2023. https://pubmed.ncbi.nlm.nih.gov/36764943/
- Corkrum et al. Neuron 2020. https://pubmed.ncbi.nlm.nih.gov/31954621/
- Durkee et al. Glia 2019. https://pubmed.ncbi.nlm.nih.gov/30801845/

SPEAKER 2

Paola Bezzi

- Petrelli et al. Biological Psychiatry 2023. https://pubmed.ncbi.nlm.nih.gov/36958999/
- Zehnder et al. Cell Reports 2021. https://pubmed.ncbi.nlm.nih.gov/33852851/
- Petrelli et al. Molecular Psychiatry 2020. https://pubmed.ncbi.nlm.nih.gov/30127471/

SPEAKER 3

Cristina García Cáceres:

- Lutomska et al. Glia 2022. https://pubmed.ncbi.nlm.nih.gov/35802021/
- Gruber et al. Cell Metabolism 2021. https://pubmed.ncbi.nlm.nih.gov/33951475/
- Garcia-Caceres et al. Cell 2016. https://pubmed.ncbi.nlm.nih.gov/27518562/

SPEAKER 4

Giovanni Marsicano

- Fernández-Moncada et al. Nature Communications 2024. https://pubmed.ncbi.nlm.nih.gov/39122700/
- Gomez-Sotres et al. Nature Communications 2024. https://pubmed.ncbi.nlm.nih.gov/39155299/
- Jimenez-Blasco et al. Nature 2020. https://pubmed.ncbi.nlm.nih.gov/32641832/

SYMPOSIUM 15

Structuring the world: noise, probabilities, chunks and contexts

Our brain seems to effortlessly infer the contents of the sensory world from the continuous stream of sensory data arriving in our senses. Yet, this is not a trivial process: the sensory data is only probabilistically associated to the elements of our environment and it is often ambiguous.

To parse the sensory data into a rich assortment of ecologically meaningful perceptual objects the brain exploits previous knowledge on the statistical structure of the sensory input. The neural processes underlying the learning of this structure and the subsequent mechanisms to parse the sensory inputs are a pressing open question in systems, human, and computational neuroscience.

In this symposium we will discuss our current understanding of how the brain chunks the sensory stream and parses it to a structured representation of perceptual objects. We will investigate the neural mechanisms responsible for this process, the neural structures and circuits implementing them, and the nature and structure of the final perceptual representation. We will address these questions from different sensory perspectives and levels of analysis, from subcortical processing of acoustic noise (de Hoz, mouse) and probabilistic stimuli (Tabas, human), to cognitive chunking (Fiser, bees, chicks, and humans), and context inference (Lengyel, computational models).

Starting with continuous acoustic noise, Dr Livia de Hoz will discuss the detection of unfolding sound level statistics across auditory sensory cortico-subcortical loops, illustrating the role of adaptation in demarking changing statistics. She will also discuss the timescales of integration across subcortical and cortical processing levels, and the role of excitatory and inhibitory cortical neurons. The discussion will be based on recent data obtained using high density electrophysiology and optogenetic manipulations in mouse auditory midbrain and primary cortex.

Prof Máté Lengyel will talk about the computational foundations of continual learning: the brain ability to learn to perform multiple tasks that require the continual creation, updating, and expression of memories. He will argue for the critical distinction between apparent and proper learning, and that contextual inference is key in continual learning. He will present predictions from computational models along with experimental data testing those predictions.

Prof József Fiser will use cross-species comparative behavioral studies involving human adults and infants, chicks and honeybees to highlight three aspects of the recent shift in perspective on what learning structured representations means. First, he will argue that a proper framework of behavioral learning should include perceptual, statistical and rule learning characteristics to form internal representations implicitly and efficiently. Second, he will demonstrate that learning the same tasks can emerge through very different computational schemes across different animals. Finally, he will highlight the complicated relationship between learning and sleep depending on the level of abstraction and the level of implicitness of the acquired knowledge.

Last, Dr Alejandro Tabas will present a series of studies in human functional neuroimaging showing how contextual sensory patterns are used to parse noisy streams of sounds to probabilistic sequences in the human auditory pathway. He will argue for a crucial role of subcortical structures in statistical learning.

Our highly interdisciplinary approach will enquire into statistical learning from a rich variety of perspectives. We will address our problem at three sensory modalities (visual, somatosensory and auditory) and two levels of processing (perception and action); with a multitude of sensory input structures from noise and textured sounds to probabilistic sequences, chunks and objects; through the lenses of complementary methodologies including neuronal electrophysiology, macroscopic neuroimaging, computational modelling, and behavioural assessments; and in four different organisms of contrasting complexities (bees, chicks, rodents, and humans). The rich multiplicity of perspectives is also reflected in the regional (our speakers are based in UK, Germany, Hungary and Spain) and career-stage (from a very junior group-leader to a research centre director) diversity.

Organisers: Livia de Hoz, Alejandro Tabas. Chair: Livia de Hoz.

SPEAKER 1

Livia de Hoz

- de Hoz L, McAlpine D. 2024. Noises on—How the Brain Deals with Acoustic Noise. Biology, 13, 501. https://doi.org/10.3390/biology13070501. https://www.mdpi.com/2079-7737/13/7/501
- Chen C, Cruces-Solis H, Ertman A, de Hoz L. 2023. Subcortical coding of predictable and unsupervised sound-context associations. Current Research in Neurobiology 5: 100110. doi: https://doi.org/10.1016/j.crneur.2023.100110. PMID: 38020811
- Chen C, de Hoz L. 2023. The perceptual categorization of multidimensional stimuli is hierarchically organized. iScience, 26: 106941. doi: https://doi.org/10.1016/j.isci.2023.106941. PMID: 3737834

SPEAKER 2

Máté Lengyel

- Lengyel G, Žalalytė G, Pantelides A, Ingram JN, Fiser J, Lengyel M, Wolpert DM (2019) Unimodal statistical learning produces multimodal object-like representations. eLife 8:e43942.
- Echeveste R, Aitchison L, Hennequin G, Lengyel M (2020) Cortical-like dynamics in recurrent circuits optimized for sampling-based probabilistic inference. Nat Neurosci 23:1138–1149.
- Heald JB, Lengyel M, Wolpert DM (2021) Contextual inference underlies the learning of sensorimotor repertoires. Nature 600:489–493.

SPEAKER 3

József Fiser

- Fiser J., & Lengyel G. (2022) Statistical learning in vision Annual Reviews of Vision 8, pp.265-290 doi.org/10.1146/annurev-vision-100720-103343
- Avarguès-Weber A., Finke V., Nagy M., Szabó T., d'Amaro D., Dyer A.G., & Fiser, J. (2020) Different mechanisms underlie implicit visual statistical learning in honey bees and humans Proceedings of the National Academy of Sciences 117 (41), pp. 25923-25934
- Garber D., & Fiser J. (2024) Structure transfer and consolidation in visual implicit learning. eLife, 13:RP100785

SPEAKER 4

Alejandro Tabas

- A Tabas, S Kiebel, M Marxen, K von Kriegstein (2024); Fast frequency modulation is encoded according to the listener expectations in the human subcortical auditory pathway; Imaging Neuroscience 2, 1-20
- A Tabas, K von Kriegstein (2023); Multiple concurrent predictions inform prediction error in the human auditory pathway; Journal of Neuroscience 44 (1)
- A Tabas, G Mihai, S Kiebel, R Trampel, K von Kriegstein (2020); Abstract rules drive adaptation in the subcortical sensory pathway; Elife 9, e64501

ABSTRACTS

INDEX

DEVELOPMENTAL NEUROBIOLOGY

INDEX

Alterations in molecular and behavioural responses to nociceptive stimuli in Mecp2-deficient CD1-mice

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Rett Syndrome (RTT) is a severe neurodevelopmental disorder caused by dominant de novo mutations in the chromosome X-linked methyl-CpG-binding protein 2 gene (MECP2). Alterations in pain perception have been reported in a great proportion of RTT individuals. Moreover, previous studies suggest that MeCP2 contributes to an early cascade of molecular events required for the initiation of pain states, and its phosphorylation (p-MeCP2) is linked to the upregulation of several nociceptive genes.

Here, we evaluated potential behavioural and cellular nociceptive alterations in an RTT murine model at symptomatic ages. Mecp2-deficient CD1-mice from both sexes, males (Mecp2-null) at 8 weeks of age and females at 6 months (Mecp2-het), were injected with capsaicin (20 µL of 3 mg/mL) to induce an acute noxious response. To establish a model of prolonged inflammatory pain, Mecp2-null received an intraplantar injection of Complete Freund's Adjuvant (CFA, 20 µL of 5 mg/mL), and nocifensive behaviours assessed using Von Frey filaments and Hargreaves apparatus for mechanical and thermal responses respectively. Brain and spinal cord tissues were collected, and immunolabelled for c-FOS and p-MeCP2 (in both WT and Mecp2-het).

Using semi-automatic tools (QUINT, ImageJ), we observed differential activation at multiple nociceptive nuclei in both Mecp2-null and Mecp2-het following capsaicin injection. Also, after the CFA model in male Mecp2-null, we observed impaired development of both thermal hyperalgesia and mechanical allodynia paired with diminished oedema development.

Our research points to alterations in nociceptive processing in Mecp2-deficient mice at symptomatic ages. Our research also demonstrates that quantifying c-FOS and p-MeCP2 with semi-automatic tools may serve to identify distinct region-specific activations. Understanding these mechanisms may contribute to a better characterization of sensory dysfunction in RTT and related disorders, with potential for the development of novel therapeutics. Supported by the Spanish Ministry of Science, Innovation and Universities (MCIN/AEI/10.13039/501100011033) and the European Union "NextGenerationEU"/PRTR (grant RYC2021-034012-I).

Analysis of nervous system-specific Rnd3 knock-out animals reveal brain cortical defects and behavioural abnormalities

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The RND3 (or RHOE) gene codes for an atypical RhoGTPase with no GTPase activity that inhibits RhoA's GTPase ability to change cell morphology and actin stress fibers. Full RND3 genetic deficiency produces postnatal lethality, profound motor deficits and neurodevelopmental delay in mice. To study specifically the role of Rnd3 in brain function, in the present study we have generated nervous-system specific RND3 knock-out animals by Cre-lox strategy. Postnatal RND3 knock-out mice often display (31.25%) hydrocephalic brains with enlarged ventricles. In some instances, the mutants display a severe phenotype (RND3severeKO) in which the ventricles that are over 30% of the brain size jeopardizing some brain structures such as the hippocampus. Surprisingly, despite these morphological defects these RND3severeKO display just small changes in their cortical structure and remain quite functional. We focused on the neuronal location by using several cortical markers to characterize differences between RND3 knock-outs and control littermates. We noticed for instance that calretinin+ neurons stay in more apical areas of RND3severeKO brains in anterior dorsal sections. Additionally, their motor ability and memory were tested. So far, results show that postnatal RND3 knock-out mice are significantly slower in speed, display a shorter stride length and have a reduced short-term memory.

Analysis of the Formation of Cranial Nerves in Human Newborns with Brainstem Dysgenesis

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Introduction: Brainstem dysgenesis is a congenital malformation that affects the development of the midbrain, pons, and medulla oblongata, causing neurological, motor, and sensory alterations due to the involvement of cranial nerve nuclei and ascending and descending nerve pathways. Studying these alterations is essential to understand their impact on neurological function and clinical manifestations.

Methods: Brainstems from several human neonates with premature death were analyzed. The samples were fixed in paraffin, sectioned with a microtome, and stained with cresyl violet, luxol fast blue, and hematoxylin/eosin to evaluate neuronal organization and morphology, identifying areas of hypoplasia or dysplasia. Additionally, immunofluorescence was applied to detect neuronal and glial markers, providing a detailed analysis of structural alterations in the midbrain, pons, and medulla oblongata.

Results and Conclusions: In the analyzed samples, the most affected cranial nerves were the oculomotor nerve (III), trochlear nerve (IV), trigeminal nerve (V), abducens nerve (VI), facial nerve (VII), glossopharyngeal nerve (IX), and vagus nerve (X). These alterations could be related to motor deficits, mastication, facial expression, and swallowing, such as ptosis, divergent strabismus, vertical diplopia, and others. The findings reinforce the relationship between brainstem dysgenesis and congenital syndromes such as midbrain hypoplasia, pontomesencephalic dysplasia, pontine dysplasia, and bulbar dysplasia. The combination of histological and immunofluorescence techniques is essential to deepen the study of these malformations and their impact on neurological function.

Analysis of the involvement of Afadin/Cdh2 in regulating proliferation and specification of cortical neural stem cells using human brain rganoids

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The neocortex is the most recent evolutionary acquisition of the brain and is responsible for complex tasks such as sensory integration and cognition. The proper neocortical organization is tightly regulated during development through precisely orchestrated mechanisms, from the generation of neural cells to the establishment of specific connections. Consequently, disruptions of this intricate process are associated with neurodevelopmental disorders, such as autism spectrum disorder (ASD) and schizophrenia.

Among the many genes implicated in regulating neocortical development, cell-cell adhesion molecules such as Cdh2 and its associated protein Afadin play crucial roles. In mice, early inactivation of these genes in the dorsal telencephalon leads to defects in radial glial cell (RGC) proliferation and differentiation, resulting in a disorganized neocortex with increased size due to an overproduction of neurons expressing upper-layer neuron (ULN) markers. Additionally, our behavioral studies revealed social deficits linked to this altered proliferation. Interestingly, mutations in Cdh2 have been described in autism patients and alterations of Cdh2 and Afadin expression have been recently found downregulated in organoids derived from ASD patients with macrocephaly.

Since ULNs are significantly expanded in primates, especially humans, and are particularly susceptible to dysregulation in neurodevelopmental disorders, we plan to use human forebrain organoids with altered expression of Cdh2 and Afadin to investigate potential defects in proliferative activity using CRISPR-Cas9. Modeling human disease in human brain organoids not only enhances biomedical relevance of the studies but also guides our research towards alternative methods that reduce reliance on animal experimentation.

APC/C-Cdh1-mediated liver sympathetic innervation regulates early postnatal body growth via IGF-1

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Neurodevelopmental diseases often course with hypothalamic neuroendocrine system impairment, which disrupts the growth hormone (GH) signaling pathway leading to hepatic inability to release insulin-like growth factor-1 (IGF-1) and to body growth retardation in early postnatal life. Those disorders are often associated with pathogenic mutations of the ubiquitin-proteasomal pathway. The E3 ubiquitin ligase anaphase promoting complex/cyclosome (APC/CCdh1) coordinates the cell cycle exit of neural progenitor cells with the onset of neurogenesis during the early embryonic period, dictating cortical size and causing perinatal lethality. Interestingly, we recently identified a human congenital heterozygous de novo mutation in the Cdh1 gene (Fzr1) that inactivates APC/C leading to postnatal microcephaly, psychomotor retardation, refractory epilepsy and body growth delay. Therefore, postnatal onset of nervous system developmental delay can cause body growth loss, although the mechanism behind this phenomenon is uncertain.
Here, we generated genetically engineering mice lacking the APC/C activator, Cdh1, in neural cells early postnatally. To do so, C57BL6/J-Fzr1flx/flx mice were crossed with mice expressing Cre recombinase governed by the nestin promoter, which is active at mid-late gestation.
We found that neural-specific Cdh1 loss caused neurodevelopmental delay, liver steatosis, and early postnatal mouse body growth loss via IGF-1 deficiency, in the absence of morphological or GH pathway functional alterations in the hypothalamus. Mechanistically, neural-specific Cdh1 loss impaired sympathetic liver innervation, leading to dysfunction of the transducers and activators of transcription-5 (STAT-5) signaling pathway, which results in liver steatosis and a failure in the transcriptional biosynthesis of Igf1 and IGF-1 protein release, causing body growth restriction.
Then, by promoting central and sympathetic nervous system development, APC/CCdh1 controls liver IGF-1 secretion to boost organismal growth through a GH pathway-independent, hepatic sympathetic nerve-mediated signaling mechanism.

Characterizing superior colliculus cell populations in sensory pathway segregation during development

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Before birth, a whisker pad stimulation in the mouse can activate both the somatosensory and visual cortices, implying that the visual and somatosensory pathways initially develop as a single system. Around birth, this multimodal integration is refined, driven by stage I spontaneous retinal activity, leading to the segregation of these pathways at the level of the superior colliculus (Guillamón-Vivancos et al., 2022). Work in the lab has shown that GABAergic neurons in the superficial layer of the superior colliculus participate in multimodal responses, which suggests that GABA signaling might be involved in shifting multimodality to unimodality. This study aims to dissect the subcortical circuits underlying this transition by investigating distinct superior colliculus cell populations, with special focus on the GABAergic cells, and mapping their connectivity with the retina and each other. To achieve this, we use electrophysiology and optogenetics in mice, both in vivo and in brain slice preparations across key developmental time points. Additionally, we employ targeted manipulations to induce and prolong multimodal responses, providing insight into the mechanisms governing sensory pathway segregation.

Clonal Mapping of the Ontogeny of the Mouse Suprachiasmatic Nucleus

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The circadian system aligns organisms' physiology in 24-h rhythms, anticipating recurring environmental changes - mainly light/dark cycles. In mammals, these rhythms are orchestrated by the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is the master pacemaker, telling every cell in the body what time it is through rhythmic signals. The 20,000 cells composing the SCN are topologically organised in the ventral core responding directly to light and the dorsal shell organizing the rhythmic output. Almost all SCN neurons produce and receive GABA, along with more than 100 neuropeptides, likely shaping the network by paracrine signalling. SCN astrocytes also play essential roles as timekeepers. Despite the well described mouse adult circuit, its ontogeny during the perinatal period remains obscure.
To investigate the mouse SCN ontogeny, we used StarTrack, a state-of-the-art cell lineage tracing tool. By in utero electroporation, we introduced at gestational day 12 a mixture of 12 plasmids encoding 6 fluorescent proteins with or without a nuclear localization signal. The plasmids randomly integrated in SCN neural progenitors labelling clones - clusters of sibling cells, derived from a common progenitor - with a permanent, unique color code. A detailed clonal analysis was then performed on sliced brains extracted from StarTrack-electroporated animals at postnatal day 30. Confocal imaging was followed by image analysis via an ad-hoc ImageJ macro. In combination, we performed an immunohistochemical mapping of core and shell neurons, oligodendrocytes and astrocytes using VIP, AVP, Olig2 and GFAP, respectively.
Remarkably, we detected clones of sibling cells throughout the SCN. With a sophisticated three-dimensional clonal dispersion analysis, we provide a high-resolution spatial reconstruction of SCN clones along the antero-posterior, dorsal-ventral and medial-lateral axes. Our results describe for the first time the mouse SCN clonal ontogeny, reporting key developmental signatures explaining the different cell types' contribution to the network.

Dmrt2 Regulates Sex-Biased Neuronal Development In The Cingulate Cortex

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Sexual differences are prevalent in the brain. DMRT transcription factors have been postulated as important determinants of sex differences. Previous research focused on the DMA subfamily in the brain. Here, we reveal an unprecedented role for Dmrt2 in regulating the proliferation and development of cortical neurons in mice. Dmrt2 is expressed in deep-layer neurons of the cingulate cortex throughout development. Its downregulation in the cingulate primordium, by in utero electroporation of shRNA, results in premature cell cycle exit of embryonic progenitors and subsequent reduction in cortical plate cellular density at later developmental stages. As development progresses, Dmrt2 expression persists in that subpopulation of neurons, controlling processes like migration, axonal targeting, and neuronal-specific gene expression. Dmrt2 expression is higher in male embryos during early development, potentially explaining their increased vulnerability to Dmrt2 depletion. This study brights to light a Dmrt2 gene function in the brain, broadens our understanding of mouse DMRTs in the nervous system and provides insights into the molecular basis of sexual differences in neurodevelopmental processes.

Dmrt3 expression could reveal a novel sexually dimorphic nucleus in the mouse hindbrain

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In mammals, numerous neural circuits interplay to modulate sensory and cognitive functions. Still, different responses to identical stimuli may emerge due to sexual differences in gene expression and connectivity within the brain. Traditionally, brain sexual differences in mammals were exclusively attributed to sex hormones, but genetic and epigenetic mechanisms might complement or balance their effect. However, the precise molecular mechanisms involved in these interactions are barely known.

The DMRT family of transcription factors (TF) emerge as conserved intrinsic regulators of sex differentiation and development of sex-specific traits throughout phylogenetically distant species. In C. elegans, some of these TFs regulate sex-specific neuronal identities, neurogenesis, synaptic pruning, and axonal morphology. However, a similar role in the mammalian brain remains to be elucidated. Here, we thoroughly describe the expression of Dmrt3 in the mouse brain from early embryonic stages throughout life by in situ hybridization (ISH) and immunofluorescence assays. With the use of molecular markers, like NKX6.1, LHX3, PAX2, and others, we identified a Dmrt3-positive cell population as V2 neurons of the gigantocellular reticular nucleus (GRN) of the hindbrain, which is mainly composed of glycinergic interneurons. Furthermore, when comparing male and female E18.5 embryos using ISH, we observed a trend towards higher counts of Dmrt3-positive cells in females compared to males. We are currently confirming that DMRT3 protein levels follow a similar trend with the use of 3D reconstructions and light-sheet microscopy. We propose that Dmrt3 could specify V2 hindbrain neuronal populations and their putative sex-specific numbers in cooperation with sexual factors. Our research will shed light onto a putative new anatomical point of sexual differences in the mouse hindbrain and the potential role of a conserved DMRT transcription factor in this process.

Early parvalbumin inhibition orchestrates long-range excitatory connectivity

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The precise interaction between excitatory and inhibitory circuits in the cortex underlies animal's complex behaviours. These two networks were believed to originate independently and from a combination of intrinsic genetic mechanisms and sensory activity-dependent plasticity. Here, we demonstrate that early inhibitory patterns in the cortex determine future long-range connectivity of excitatory neurons.

We first employed chemogenetic tools to specifically alter L2/3 pyramidal neuron activity during early postnatal development. DREADD-dependent inhibition of L2/3 targeted cells increases the number of interhemispheric projection neurons and the axons innervating the contralateral cortex in the adult. These changes are prospective since no phenotypes were observed at short-term stages upon activity manipulation. We also described a developmental window of plasticity given that later interventions did not alter L2/3 wiring.

We further showed that early manipulation in the activity of parvalbumin (PV) GABAergic interneurons changed the wiring decisions of L2/3 pyramidal neurons and the maturation of adult long-range interhemispheric connectivity. We applied cutting-edge AI-based 3D analyses and quantified, in thousands of individual neurons, the early structural synaptic reorganization that could explain the observed rewiring phenotypes. After PV activity manipulation, we reported significant plastic changes in the development of PV-specific inhibitory inputs and thalamocortical innervation onto L2/3 neurons.

Our data provide significant insights into how long-range cortical connectivity is assembled and refined, and suggest circuit-level mechanistic explanations in neurodevelopmental disorders, where concomitant alterations in inhibitory activity and the structure of excitatory circuits are widely reported.

EFFECTS OF LIS1 MUTATION ON THE POSTNATAL DEVELOPMENT OF PARVALBUMIN-POSITIVE INTERNEURONS IN THE CINGULATE CORTEX

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Numerous neurological pathologies are characterized by an imbalance between excitatory and inhibitory signaling within brain microcircuits. The loss of function or degeneration of parvalbumin-positive (PV+) cortical GABAergic interneurons, is associated with network disruption and cognitive dysfunction in various neuropsychiatric disorders. Platelet-activating factor acetylhydrolase 1B subunit alpha (Lis1) is a key regulator of dynein-mediated motility, mitotic nuclear positioning, and microtubule organization. Mutations in the Lis1 gene have been linked to lissencephaly in humans, as well as defects in neural migration and epilepsy. To investigate the structural consequences of Lis1 silencing in PV+ interneurons, we have developed a transgenic animal model in which this gene is specifically deleted in PV+ interneurons. We examined the number of PV+ interneurons in the cingulate cortex, encompassing both the anterior and retrosplenial cortices. Our findings reveal a significant reduction (48%) in PV+ interneurons in the cingulate cortex of Lis1 mutant mice compared to controls at postnatal 21 days, with marked decreases in cortical layers II/III, V, and VI (51%, 47%, and 35%, respectively). These results suggest impaired migration, maturation, and/or survival of PV+ interneurons. Notably, the decrease in PV+ interneurons appears to begin after postnatal day 15, indicating that the disruption of interneuron survival likely starts at this time. Interestingly, the reduction in PV+ cells appears to be specific to this subtype of interneurons, as other interneuron populations, such as somatostatin-positive cells, remain unaffected. Additionally, we used three-dimensional immunohistochemistry (iDSICO protocol) to analyze the activity (cFos labelling) in the whole brain postnatally and we observed an increase of the number of cFos expressing neurons in the anterior cingulate area, neurons that did not coexpressed PV. Further studies are required to elucidate the underlying mechanisms driving these effects and to evaluate the functional consequences. Funding: MCIN grants-PID2020-118171RB-I00 and PID2023-150680OB-I00; GVA-Prometeo Grant-CIPROM/2021/018.

Expression of Hedgehog signalling genes in the juvenile shark retina suggests a role in postnatal neurogenesis

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In the postnatal fish retina, neurogenesis persists in two main neurogenic niches: Müller glia and the ciliary marginal zone (CMZ). In the retina of the small-spotted catshark (Scyliorhinus canicula), neurogenesis also persists postnatally, although mitotic activity becomes nearly absent in adulthood. Bulk RNA sequencing comparing juvenile and adult retinas showed changes in the expression of genes related to Hedgehog signalling, including increased expression of hedgehog-interacting protein (hhip, an inhibitor of Hedgehog signalling) in adults, and decreased expression of sonic hedgehog (shh) and gli2 (a transcription factor that mediates Hedgehog signalling), suggesting that Hedgehog signalling is reduced in the adult shark retina. Furthermore, single-nucleus RNA sequencing analysis of the juvenile retina showed expression of key Hedgehog pathway genes (ptch1, ptch2, smo, gli2, gli3, hhip) in both retinal progenitor cells and Müller glia. To validate these results, we studied the expression of shh and gli2 in the juvenile retina of S. canicula using immunohistochemistry and in situ hybridization techniques, and we observed expression in both retinal progenitor cells and Müller glia. Taken together, these results suggest a possible involvement of the Hedgehog pathway in regulating postnatal neurogenesis in the juvenile retina of S. canicula. In light of these data, we performed intravitreal injections of cyclopamine (Hedgehog signalling antagonist) and SAG (Smoothened agonist) in juvenile specimens. Quantitative PCR of treated versus control (DMSO injected) retinas showed changes in the expression of Hedgehog pathway genes, confirming the effectiveness of the treatments. Further studies are needed to confirm the specific roles of Hedgehog pathway genes in postnatal retinal neurogenesis in sharks.

Functional characterization of enhancers controlling the expression of human neurodevelopmental genes in organoid models

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Brain cortex development hinges upon the activity of genes exhibiting dynamic and tissue/cell-specific expression patterns. Typically, their transcription is governed by combinations of cis-regulatory elements (CREs) such as enhancers, repressors, and silencers. Mutations in CREs are associated with congenital brain defects and human brain evolution.
Although the availability of chromatin epigenetic profiles has facilitated the identification of potential CREs on a genomic scale, their functional characterization requires intricate functional genomic assays, often challenging to execute in animal models .
We address this challenge by using human brain organoids as model system and developing novel recombination- mediated enhancer transgenesis approaches exploiting the properties of the PhiC31 or Bxb1 integrases . We compared the efficiency of the PhiC31 and Bxb1 recombinases for the integration of constructs in human iPS cells and tested the activity of candidate regulatory sequences in organoids cultures derived from the obtained transgenic lines.
Combining epigenetic, chromatin 3D architecture and GWAS databases we identified candidate regulatory sequences that: (1) exhibit active enhancer epigenetic marks in the human foetal cortex, (2) are located in the regulatory domain of known neurodevelopmental genes, and (3) are associated with polymorphisms described in patients with brain disorders/malformations. Particularly, we focused on three candidate enhancers of the developmental TF FOXG1, a key player in the development of forebrain structures, such as the dorsal and ventral telencephalon and the retina .
We evaluated the activity of all these elements in cortical, retinal and ventral forebrain organoids, showing that two of the three display enhancer activity in our assays.
Our findings suggest that combining our recombination transgenesis approaches with the use of region-specific brain organoids may offer a potent approach to characterize the activity of these candidate enhancers and the effect of their associated pathological mutations.

Hippocampal function alterations in mouse models of Lamb-Shaffer syndrome

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Lamb-Shaffer Syndrome (LAMSHF ; OMIM #616803) is a rare genetic disorder caused by mutations in the SOX5 gene, affecting multiple organ systems. Many of the manifestations in LAMSHF patients are of neurological origin, including intellectual disabilities, generalized delays in speech and learning, anxiety, and behaviors associated with Autism Spectrum Disorders (ASD), such as social deficits and stereotypical behavior.
Understanding the pathophysiology of this complex syndrome demands the development of robust animal models to study the individual neural components involved. In this study, we examined hippocampal function using two distinct LAMSHF mouse models: i) a conditional loss-of-Sox5 model during embryonic development specifically in the CA2 region of the hippocampus (Amigo2-cre/Sox5fl/fl; Sox5Amigo2), a region crucial for social memory; and ii) a tamoxifen-inducible conditional loss-of-Sox5 model specific to the central nervous system (Sox2-creERT2/Sox5fl/fl/YFP+; Sox5icKO), which enables Sox5 depletion in neural stem cells (NSCs) of the subgranular zone of the dentate gyrus, responsible for adult neurogenesis maintenance, a process crucial for spatial memory. By combining cellular characterization strategies and transcriptomic tools with an extensive battery of behavioral assays, we found that: i) Sox5Amigo2 mice exhibited alterations in social memory related to ASD traits, and ii) Sox5icKO model displayed impaired adult neurogenesis, leading to deficits in spatial and contextual memory, both common features in LAMSHF . These findings provide valuable insights into the neurological mechanisms underlying the syndrome and highlight the potential of these mouse models for the future exploration of therapeutic approaches.

Human 2D cell culture and 3D brain organoid models to study the effects of NF1 mutations on neurodevelopment

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RASopathies are caused by mutations in genes encoding Ras or associated proteins of the MAPK pathway, representing the most common group of neurodevelopmental disorders (1 in 1,000 newborns). Neurofibromatosis type 1 (NF1), one of the most prevalent syndromes in this group, is an autosomal dominant disorder caused by loss-of-function mutations in NF1 gene, which encodes neurofibromin, a negative regulator of the Ras/MAPK pathway. Patients with NF1 usually present café-au-lait macules, bony anomalies, intellectual disability, and increased risk for cancer and autism spectrum disorder. Despite its relatively high prevalence, the pathophysiology of NF1 remains unclear. The aim of this research project is to study the impact of different NF1 mutations on neurodevelopment using emerging human-derived induced pluripotent stem cell (iPS) models, including 2D neuronal/glia cultures and 3D brain organoids.
Using these models, we have analysed multiple neurodevelopmental features in iPS from healthy controls and several NF1 patients throughdifferent techniques (Western blot and immunofluorescence). We studied neural progenitor proliferation, neuron/glia specification, migration, and neuronal differentiation (including neurite outgrowth and synapse formation), using molecular markers. Additionally, we have studied functional neural network dynamics by calcium imaging. Furthermore, we have determined the activity of ERK and Akt proteins downstream of Ras at different neurodevelopmental periods to better understand the role of these signalling proteins in NF1.
To summarize, this project takes advantage of novel human cellular models to gain insights into the effects of NF1 mutations on brain development. Thus, the 2D model simplifies the study of neurodevelopment, facilitating detailed analysis of cellular differentiation. On the other hand, the 3D model reveals important structural aspects of the brain related to its architecture, organization and connectivity. By combining these two human cell models, we expect to gain insights into NF1 brain pathophysiology that could contribute to a better understanding of this rare genetic disease.

Human midbrain organoids, a model to study the role of DMRTA1 and DMRTA2 genes in the genetic susceptibility to ADHD

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Recent studies have revealed different roles of DMRT (doublesex and mab-3 related transcription factors) genes in brain development and sexual differentiation across phylogenetically distant species. Genetic studies from the lab have linked DMRTA1/Dmrt4 and DMRTA2/Dmrt5 to Attention-Deficit/Hyperactivity Disorder (ADHD). We hypothesize that DMRTA1 and DMRTA2 influence ADHD through sex-specific mechanisms, with implications for brain development and neuropsychiatric conditions.
GWAS studies identified genetic associations between regulatory variants in these genes and ADHD. Neuroimaging shows that DMRTA1 affects cortical regions involved in cognitive control, and DMRTA2 regulates brain areas linked to attention regulation. DMRTA1 and DMRTA2 are important for cortical and midbrain (Mb) development. DMRTs regulate neuronal identity, proliferation, and connectivity. Dmrt5 is expressed in ventral midbrain progenitors, where it regulates neuronal differentiation and, in parallel, inhibits sexual differences in Mb development. Dmrt4 is transiently expressed in post-mitotic midbrain dopaminergic neurons, but in Dmrt5 knockout (KO) mice, Dmrt4 is ectopically expressed in Mb progenitors, suggesting an intricate regulatory network between DMRTs in Mb sexual development.
To investigate these genes in human neurodevelopment, we use cortical (hCO) and midbrain (hMO) organoids derived from induced pluripotent stem cells (iPSCs). We assess their regional identity with molecular markers and have observed key dopaminergic markers in midbrain-patterned organoids, indicating developmental progress. We are also generating DMRTA1 and DMRTA2 KO iPSC lines using CRISPR/Cas9 to explore their roles in neuronal differentiation, lineage specification, and sex-dependent regulatory mechanisms.

Impact of Lis1 Mutation on Somatostatin-Positive Interneurons in the Hippocampus and Cingulate Cortex

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Approximately a quarter of all neurons are inhibitory interneurons, with somatostatin-positive interneurons (SST+ interneurons) forming a distinct subtype that expresses both somatostatin and GABA. These cells are essential for modulating cortical and hippocampal information processing, playing a pivotal role in maintaining the excitation/inhibition balance by synapsing with pyramidal neurons and other interneurons. In our study, we focused on the expression of somatostatin in the hippocampus, specifically in the CA and hilus regions, and the cingulate cortex. In addition, we have previously conducted studies examining the tangential migration of SST+ interneurons, where we observed a significant reduction in the migratory population. Our findings revealed a marked decrease in SST+ interneurons both in CA regions and the hilus of hippocampus, as well as in cingulate cortex, suggesting a disruption in the migration and subsequent settling of these interneurons. This reduction in SST+ interneurons likely contribute to alterations in local inhibitory circuits, leading to imbalances in excitation and inhibition. These changes could impact cognitive functions such as spatial memory, learning, and higher-order processing. Further investigation is needed to explore the underlying mechanisms driving this reduction in SST+ interneurons and to assess their broader functional implications on neural circuits and behavior.

Implications of Afadin in the Organization and Functionality of the Ventricular-Subventricular Zone

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The ventricular-subventricular zone (V-SVZ) of the lateral ventricles is one of the neurogenic niches in the adult mammalian brain. Neural stem cells (NSCs) residing in this niche are embryonically pre-specified and remain quiescent until their postnatal reactivation, at which point they generate interneurons that migrate through the rostral migratory stream (RMS) to the olfactory bulb (OB). Multiple mechanisms contribute to the precise regulation of the transition between quiescent and activated states of NSCs. Some of these mechanisms involve signals from the environment or neighboring cells through cell adhesion molecules (CAMs), such as cadherins.
Previous studies from our group reported an expansion of embryonic cortical progenitors following the conditional deletion of Cadherin-2 (Cdh2) or one of its adapter proteins, Afadin, in the dorsal telencephalon. This early inactivation disrupts adherens junctions between NSCs causing over-proliferation and severe malformations, including hyperplasia and “double cortex” formation. However, while the role of Cdh2 in regulating postnatal neurogenesis has been described, the potential involvement of Afadin in this process remains elusive.
In this work, we investigate Afadin’s involvement in the establishment and proper function of the postnatal V-SVZ niche by analyzing the consequences of its perturbation during early development and postnatal stages. Our results reveal significant alterations in V-SVZ and RMS architecture, as well as increased proliferation and neurogenic output, suggesting that Afadin plays a role not only in the structural organization of the niche but also in maintaining the homeostasis of adult neurogenesis.

Indirect pathway linage-specific alterations from early development in Huntington's Disease

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Huntington's disease (HD) is an incurable neurodegenerative disorder characterized by motor and cognitive impairments due to a predominant loss of striatal projection neurons (SPNs). Growing evidence suggests that developmental abnormalities may predispose specific neuronal populations to degeneration, particularly SPNs. Understanding these early alterations is critical to further understand disease mechanisms and progression.

Here we show an early HD-related expression pattern elucidated by bulk RNA-seq performed on wt and HD isolated striatal primordia at embryonic day 16, combined with histological analysis. Our results reveal that early alterations in cell cycle progression and cell fate determination in HD embryos give rise to an imbalance between specific neural precursor cells and mature neurons. We show that the neurogenesis of the indirect SPNs (iSPNs) linage is advanced, generating more progenitors at embryonic stages. We also observed that the excess of iSPNs generated undergoes into specific apoptosis, balancing the final numbers of iSPNs. In addition, we have evidences of the specific neural progenitor cell subpopulation that produces this aberrant neurogenesis at early embryonic stages.

In conclusion, here we show that a specific alteration in subsets of striatal neural progenitor cells can be the cause of developmental alterations observed in HD, producing an early imbalance of SPN. Identifying these pathways is crucial to develop new strategies to restore neuronal homeostasis, protect striatal neurons and prevent HD progression.

This study was supported by grants from the Ministerio de Ciencia e Innovación (PID2021-126961OB-I00, and PDC2022-133783-I00); Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación and European Regional Development Fund (ERDF A way of making Europe) (Red de Terapias Avanzadas, RD21/0017/0020, and RD24/0014/0016); European Union NextGenerationEU/PRTR; Generalitat de Catalunya (2021 SGR 01094); and "la Caixa" Foundation under the grant agreement LCF/PR/HR21-00622 and LCF/BQ/PI24/12040007, Spain.

Investigating the molecular mechanisms regulated by Afadin in the control of neocortical stem cell proliferation and specification

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The neocortex is a critical brain structure responsible for higher cognitive functions in mammals. Its proper function and structural integrity depend on the generation and organization of an appropriate number of excitatory and inhibitory neurons, along with other regulatory cells. Neural stem cells, known as radial glial cells (RGCs), give rise to this cellular diversity during embryonic development and a precise balance between their proliferation and differentiation is essential for this process. Among the many genes involved in regulating these cells during development, cell-cell adhesion molecules such as Cdh2 and its associated protein Afadin play fundamental roles. Inactivation of these genes in the dorsal telencephalon at early developmental stages leads to defects in RGC proliferation and differentiation, resulting in a disorganized and enlarged neocortical structure with an overproduction of neurons expressing upper-layer neuron markers. Additionally, behavioural characterization of Afadin cortical conditional mutant mice has revealed social deficits. Notably, the alterations mentioned resemble those observed in certain murine models of autism spectrum disorder (ASD) and some ASD patients.
In this study, we analyzed gene expression changes using RNA sequencing and quantitative PCR, alongside histological assays, in Afadin cortical conditional mutant mice at E13.5. Our findings provide insights into the molecular mechanisms by which Afadin regulates neocortical proliferation and neural specification, contributing to a better understanding of its role in proper neocortical development.

Keywords: Afadin, neocortex development, radial glial cells

Mapping the reptilian pallium: cellular and structural differences between turtles and geckos

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The most dorsal region of the telencephalon, the pallium, is crucial for key functions such as learning, memory, and social behaviour. Its functional diversity, complex anatomy, and extensive connectivity make it a central focus in evolutionary neuroscience. However, the number of histogenetic domains and the cellular composition of this region in vertebrates remain debated. Reptiles provide a valuable model for studying pallial evolution and the ancestral phenotypes of amniote brains.
In this study, we analyze the expression of various cellular markers of the adult pallial region in two reptilian species, the turtle Trachemys scripta elegans and the gecko Paroedura picta, using immunohistochemistry, hybridization chain reaction, and 3D light-sheet fluorescence microscopy.
We identified two distinct regions in the dorsal cortex (DCx) of both species based on Satb1/Satb2 expression, a finding significant because this DCx subdivision had previously been reported only in crocodiles. Additionally, turtles exhibit a prominent pallial nucleus along the rostrocaudal axis, known as the pallial thickening, whereas in geckos, this nucleus is confined to rostral regions and is less prominent, suggesting a more limited evolutionary development of the pallial thickening in lepidosaurs. Conversely, geckos possess the sphericus nucleus (Ctip2+), absent in turtles, which serves as a key structure for vomeronasal processing. Other structures, such as the medial, dorsomedial, and lateral cortices, as well as the dorsal ventricular ridge, exhibit similar cellular characteristics in both species, indicating that the cells within them may have been present in the last common ancestor of amniotes.
The application of 3D light-sheet fluorescence microscopy allowed for unprecedented visualization and comparative analysis of these structures along the rostrocaudal axis. This is the first use of this technique in the reptilian pallium, highlighting its potential for evolutionary neuroanatomy. Our findings provide new insights into pallial diversification and amniote telencephalic evolution, encouraging further comparative and functional studies.

Meis2, evolutive analysis and expression patterns in the developing Xenopus laevis brain

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The Meis2 gene, a member of the TALE homeobox family, has been sequenced in many vertebrates and is known to play a crucial role in mammalian embryonic development, particularly in the formation and function of the central nervous system. The present study first examined the amino acid sequence evolution of this transcription factor in key amniote and anamniote vertebrates in phylogenetic diversification. This was followed by an analysis of its expression pattern in the anuran amphibian Xenopus laevis developing nervous system, a key evolutionary representative at the basis of anamniote-amniote diversification. We analyze our results in this anamniote tetrapod in comparison with those previously described in amniotes, highlighting conservative features and specializations in vertebrate neural development and brain regionalization.

Metabolic control of adult neural stem cell quiescence

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Adult Neural Stem Cells (NSCs) exist in different states of activation that go from a quiescent non-proliferative (qNSCs) to an actively proliferating (aNSCs) state[1]. Knowing the factors responsible for NSCs activation could be a promising strategy to modulate neurogenesis. One of the factors modulated during NSCs activation is metabolism. The quiescence state relies on glycolysis and fatty acid oxidation, whilst aNSCs are more dependent of oxidative phosphorylation[2,3]. In this work, we studied the role of glycolysis for the induction and maintenance of quiescence in adult NSCs of the subependymal zone (SEZ). To approach this, we have used a conditional knock-out mouse to eliminate 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3LoxP/LoxP), an enzyme that regulates the flux of glycolysis[4], in a cell-specific manner. AdV-mediated delivery of a Cre recombinase to primary cultures of neurospheres from adult PFKFB3LoxP/LoxP mice resulted in a downregulation of glycolysis that rendered NSCs more proliferative. To study the relevance of glycolysis in qNSCs in vivo, we electroporated early postnatal PFKFB3LoxP/LoxP mice with episomal non-integrative plasmids, only retained in qNSCs, carrying GFAP-CreERT2. Recombination following tamoxifen injection at 2 months of age resulted in an increased number of aNSCs, neuroblasts, and proliferating cells in the SEZ of PFKFB3 knock-down animals. These results indicate that the glycolytic metabolism characteristic of qNSCs is necessary for the maintenance of the quiescent state.

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Metabolic post-translational modifications regulate adult neural stem cell quiescence

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Neural stem cell (NSC) quiescence is not a passive default state but a tightly regulated process influenced by niche-derived signals, cell-cell interactions, and extracellular matrix components. Transcriptomic analyses have identified metabolism as one of the most dynamically altered pathways during NSC activation, suggesting that metabolic cues may function not only as energy sources but also as key regulators of gene expression and protein function. Here, we investigate the role of two metabolism-associated post-translational modifications (PTMs)—O-GlcNAcylation, linked to the hexosamine biosynthetic pathway (HBP), and lactylation, derived from glycolysis—in the maintenance of NSC quiescence. Using primary cultures of adult NSCs and in vivo characterization of the neurogenic lineage, we demonstrate that both PTMs promote NSC quiescence by reducing proliferation without compromising cell viability. Furthermore, targeted proteomic analysis revealed differentially modified proteins, identifying potential candidates involved in the metabolic control of NSC fate. Our findings suggest that these PTMs act as metabolic checkpoints regulating NSC activation, providing new insights into the interplay between metabolism and neural stem cell dynamics.

Microglial electron transport chain sustains brain development with different roles for each complex

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Microglia are the brain innate immune cells with relevant functions during development, adult maintenance, and the progression of brain diseases. Microglial function is highly dependent on metabolic adaptations. Previous data suggest that similar to monocyte-derived cells, microglial activity depends on a metabolic switch from aerobic (dependent on the mitochondrial oxidative phosphorylation system –OXPHOS–) to anaerobic glycolysis. However, we and others have shown that microglial activity is correlated with the transcriptional upregulation of OXPHOS, suggesting their requirement for normal microglial activation during the critical developmental period or the activation associated with neurological disorders. To further investigate the role of OXPHOS in microglial function during development, we generated mouse models deficient for either Complex I or Complex III activity in microglia (MGcCI or MGcCIII) from the embryonic stage. Previously (Mora-Romero et al., 2024), we characterized the MGcCI mouse model and demonstrated that the lack of CI in microglia strongly reduces ATP levels and increases NADH, suggesting reductive stress. Remarkably, MGcCI microglia is highly active at the transcriptional level, passing from a functional state at juvenile age (1-month old) to a dysfunctional state at adult age (3-months old). Moreover, MGcCI mice develop behavioral deficits and early lethality at 3-months old. To better understand the mechanisms underlying these phenotypes, we generated the MGcCIII mouse model. As expected, MGcCIII microglia show a stronger ATP reduction than MGcCI microglia but a milder decrease of the NADH levels. However, MGcCIII microglia do not show signs of dysfunctionality until 10-months of age, when animals start to develop behavioural problems and start to die prematurely. Therefore, we propose that there might be additional mechanisms beyond the lack of ATP that explain the stronger/earlier phenotype observed in MGcCI animals compared to MGcCIII model. To decipher these mechanisms, we are currently conducting a battery of experiments including metabolomics and epigenomics assays.

Molecular characterisation of progenitor cells and their fate potency in the postnatal shark retina

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In the mammalian retina, neurogenic activity is generally thought to be restricted to embryonic and early postnatal stages, while retinal neurogenesis persists well into postnatal life in other vertebrates. In the postnatal fish retina, new neurons are generated by two main neurogenic niches: the ciliary marginal zone (CMZ) and Müller glia. In teleosts, the CMZ is able to generate all retinal cell types except for rod photoreceptors, which derive from Müller glia. In sharks, although neurogenic activity is known to be present in the postnatal retina, the ability of postnatal retinal progenitors to give rise to different retinal cell types (i.e. their fate potency) is unknown. To address this, we used a 5-ethynyl-2'-deoxyuridine (EdU) labelling pulse-chase strategy in combination with the immunohistochemical detection of various markers of mature retinal cells in juvenile small-spotted catsharks (Scyliorhinus canicula). This showed newly generated rod photoreceptors both adjacent to the CMZ and scattered throughout the central retina, suggesting that rods are derived from Müller glia, resembling the teleost retina, although further work is needed to confirm whether rods are also generated by the CMZ. To gain further insight into this, we analysed a single-nucleus RNA sequencing atlas of the juvenile retina (previously published by us), where several populations of retinal progenitors, photoreceptor precursors and rod photoreceptors were identified. Pseudotime analyses allowed us to reconstruct the developmental trajectories of rod photoreceptor generation in the postnatal shark retina, identifying genes potentially involved in the regulation of this process. Our results suggest a conserved mode of postnatal rod photoreceptor between teleosts and cartilaginous fishes, which could be ancestral to all jawed vertebrates. Further comparisons with other taxa will allow us to better understand the evolution of postnatal neurogenesis in the vertebrate retina.

Neddylation is essential for oligodendrocyte and astrocyte development and for the myelination process

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Myelin sheaths are, concentric wraps of glial membrane that form compact lipid-rich lamellae around axons, generated by oligodendrocytes (OLs). Myelin is essential not only to provide crucial metabolic support to axons but also to increase the action potential conduction velocity. Astrocytes are other key players involved in CNS myelination, among their other functions by facilitating OLs differentiation and mediating OL–axon initial contact. Most research has focused on elucidating the transcriptional mechanisms involved in myelination, whereas post-translational (PTM) mechanisms have barely been studied. Recent studies have described neddylation, a PTM analogous to ubiquitination, as involved in numerous cellular functions including cell division and differentiation, apoptosis, and proteolysis. Although its impact on neuronal development has been documented, its impact on CNS glial development remains unknown. Using different techniques, such as Western Blot, qPCR, immunostaining, and RNA-seq and pharmacological and genetic inhibition of neddylation, we have demonstrated that neddylation is essential for OL differentiation and maturation. Furthermore, in experiments to define the mechanisms by which neddylation exerts such profound effects on OL myelination, we performed RNA-seq profiling of OLs cultured in the presence or absence of MLN4924 (a specific neddylation inhibitor). We identified pathways that could be involved in regulating the neddylation-mediated effects on myelination, including WNT pathways or the master transcription factor Myrf. In parallel, experiments in cultured astrocytes showed, that acute and chronic treatment with MLN4924 activate astrocytes by increasing the number of amoeboid astrocytes, altering their morphology, and inducing cell death. Finally, their migratory capacity was diminished, which is essential for the myelination process. Thus, we have solid data demonstrating that neddylation is crucial for OL differentiation and maturation, for development of astrocytes, and for the myelination process.

Neurodevelopment-associated transcription factors in the maintenance of mature neurons

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Neurons are not renewed throughout the lifespan. Over time, these cells accumulate damage due to the high metabolic demands required for the maintenance of identity and precise circuit connectivity. Thus, across evolution, mechanisms to ensure neuronal homeostasis and long-term survival have been established. Cux1 is a homeobox transcription factor highly conserved in metazoans which mediates the acquisition of neuronal identity and connectivity of cortical neurons through the regulation of gene expression and nuclear matrix attachment. In mice, Cux1 is composed of 33 exons; large genes are more likely to undergo oxidative stress- and metabolic- induced somatic mutations. Besides, brain of patients with age-related neurodegenerative diseases shows mutations in CUX1. Therefore, we consider Cux1 as a perfect candidate to understand its roles in late developmental scenarios.

We used an inducible Cux1 knock-out mouse model in cortical neurons at postnatal day (P) 21, when developmental circuit assembly is established. We observed that Cux1 depletion alters DNA nuclear distribution at P31, a phenotype recovered at P37 by a subset of neurons. However, non-recovered neurons undergo abnormal levels of cell death between P31 and P37. In parallel, we detect an aberrant hyperactivation of microglia and engulfment of neuronal debris between these two time points. These results conclude that neuronal survival is compromised upon Cux1 late mutation and suggest a narrow temporal window of vulnerability given that we cannot detect any further death after P37. To evaluate the behavioral consequences after Cux1-dependent neuronal death, we tested whisker-based discrimination at later time points (i.e. P60) and found deficits in sensory processing, suggesting that the subjacent circuit might be severely affected.

Our findings expand Cux1 functions not only as an important determinant during development, but also as a housekeeper for the homeostatic maintenance of mature neurons.

Neurohistological study of the LIS-1 mutation in CamkIIa expressing Purkinje Cells in the mouse Cerebellum

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The human brain's development is a marvel of precision, where genetic blueprints orchestrate the proliferation, migration, and differentiation of neurons. However, neurodevelopmental disorders like lissencephaly (LIS1) disrupt these processes. LIS1's cortical focus has overshadowed the forgotten cerebellum so the aim of this study is to shed light on Lis1's specific impact on this region.
To investigate this subject, we generated specific conditional Lis1 knockout-RFP mice (Lis1 cKO-RFP under CamkIIa-Cre driver; a Calcium/Calmodulin-dependent protein kinase II alpha, and a specific enzyme of the Purkinje Cell (PC) population) inducible by Tamoxifen. The induced mutation was performed in P0 mice and brains were collected and analyzed at P21 by means of immunohistochemistry stainings and stereologically quantified using Neurolucida and Stereo Investigator Software (Mbf Bioscience @). Under these conditions we found that the major mutation effects occurred in the posterior cerebellar folia, mainly the uvula (IX) and the nodulus (X). In these folia, most of the PC neurons were either misplaced in the granular cell layer or misaligned within the Purkinje cell layer, disrupting the typically well-established monolayer, characteristic of this cell type in the cerebellum. Their morphology was also altered, showing a distorted and tilted distribution of the initial dendrite segment and dendritic arborizations relative to the cerebellar surface. Similar alterations were observed regarding the position and shape of the PC somas in respect to the initial dendrite segment. We further investigated the possible alterations in their local synaptic connectivity of the mutated Purkinje cells and intrinsic cerebellar circuitry. These results demonstrate that LIS1 plays a critical but often underestimated role in cerebellar development, and circuit formation, and adds another perspective into the phenotypic alterations observed in human subjects.

Pax6 isoforms label distinct stages of neuronal differentiation in the mouse brain

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The transcription factor Pax6 is a key regulator of complex developmental processes in both the embryonic and adult brain. During early development, it is widely expressed across the eye, telencephalon, diencephalon, and midbrain, marking both progenitor and differentiated cells. In the adult brain, Pax6 expression persists in neural stem cells within neurogenic niches, as well as in mature neurons of the olfactory bulb and piriform cortex, and specific astrocyte populations. Interestingly, overexpressing Pax6 in mature astrocytes can reprogram them into progenitor-like cells, reinforcing its role in neural stem cells. However, in mature neurons, Pax6 endogenous expression does not alter their differentiated state, suggesting the presence of cell-type-specific regulatory mechanisms governing its function. How can the same gene perform such different roles in progenitor cells compared to mature neurons? While cellular context could lead to different responses to the same gene, a simpler explanation is alternative splicing. This process generates variants (isoforms) of the same gene with distinct structures and functions. Pax6 produces three major isoforms, each differing in their DNA-binding properties; however, their precise contributions to Pax6's functional diversity remain largely unexplored. To define their specific role, we used CRISPR to engineer different mouse knock-in lines in which each isoform is tagged with a short epitope, enabling their individual detection. Our findings revealed a dynamic shift in isoform expression during neuronal differentiation: progenitor cells predominantly express the canonical Pax6 isoform, but as differentiation progresses, its levels decline, while Pax6(5a) becomes the dominant variant in mature neurons. These results suggest a functional transition, where the canonical Pax6 isoform supports proliferation, while Pax6(5a) plays a role in differentiation and neuronal identity maintenance. This shift in isoform dominance sheds light on how Pax6 regulates neuronal fate, opening new avenues for understanding its broader role in brain development, cell therapy and neurodevelopmental disorders.

Perinatal Emergence of Cerebellar Spontaneous Activity and Its Role in Long-Range Connectivity

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The development of the cerebellum and its connections is a critical process that spans from embryonic to early postnatal stages. Cerebellar disruptions during this crucial window are frequently linked to neurological impairments, affecting motor, cognitive, and social behaviors. Despite significant progress in understanding cerebellar specification and function, the precise mechanisms underlying the development and organization of cerebellar neuronal subsets into functional brain circuits remain poorly understood. A comprehensive characterization of early cerebellar activity patterns and how their perturbation may impact cerebellar circuitry is particularly lacking. This is a crucial question, as transient disruptions in activity during critical developmental windows can induce lasting changes in structural and functional connectivity, potentially contributing to the onset of complex pathophysiological conditions originating in the cerebellum.
In this study, we investigate the emergence of spontaneous calcium activity in the developing cerebellum. Using transgenic mouse models and advanced imaging techniques, we characterize distinct cerebellar activity patterns during embryonic and early postnatal stages. Our findings highlight a key role of the cerebellar nuclei (CN) in this early rise in activity and reveal dynamic shifts in activity patterns from embryonic to perinatal stages. These transitions suggest a potential role in establishing cerebellar circuits during early postnatal development. Given the CN's function as the primary output of the cerebellum, we further assess whether alterations in this perinatal activity affect long-range connectivity. Our findings reveal that disrupting this early activity impairs the precise wiring of these connections, underscoring its essential role in both the development and maintenance of cerebellar efferent connectivity.
In conclusion, defining the spatiotemporal dynamics of spontaneous cerebellar activity patterns provides critical insights into the mechanisms shaping early cerebellar development. These findings open new perspectives on how early cerebellar activity may shape both cerebellar and extracerebellar circuits, ultimately contributing to the maturation of global brain function.

Photic input and development of the mouse suprachiasmatic nucleus

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The suprachiasmatic nucleus (SCN), the master clock – is the brain region that regulates circadian rhythms and allows mammals to anticipate daily changes in the environment. The SCN ensures synchronization of physiology and behavior to day/night transitions. As the SCN receives direct innervation from the retina, light can reset the master clock. Although the SCN response to light is well described in adult mice, it is still unknown how circadian rhythms emerge during perinatal development and what is the role of photic time cues in the process.

In order to understand SCN ontogeny, we have sequenced with single-cell resolution SCN samples at critical timepoints during development and at maturity. With the expression of well-known cell markers, we clustered the different cell types that form the SCN. Applying an unbiased approach, we assessed cell type-specific changes along development based on gene expression patterns. We focused on cell-type specific developmental changes related to light reception and maturation of this almost entirely GABAergic circuit where inhibitory transmission is critical. In line with this, we complemented the snRNA-seq analysis with an in-depth immunohistochemical characterization of the expression pattern of KCC2 along the developmental trajectory. KCC2, the neuron-specific K+-Cl- cotransporter-2, maintains low intracellular chloride levels in mature neurons, which are necessary for the inhibitory effect of GABA.

Interestingly, we found for the first time that KCC2 expression increases with neuronal maturation in the light-receiving part of the SCN. To understand how light can affect this developmental feature, we compared the postnatal development and KCC2 expression in mice born from mothers housed either under a normal light/dark (L12/D12) cycle or in constant dark (DD) conditions. Our findings are critically important to understand SCN postnatal development in mice by exploring how light during early life can impact the maturation of the SCN circuit in a cell type-specific manner.

RhoE/Rnd3: molecular mechanisms involved in the formation of the cortex during mouse brain development

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RhoE/Rnd3: molecular mechanisms involved in the formation of the cortex during mouse brain development
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The knowledge of the molecular and cellular mechanisms that are involved in brain development is rather limited. Moreover, while many extracellular cues and specific receptors have been shown to play a role in different aspects of nervous system development, including neuron migration and axon guidance, examples of intracellular signalling effectors involved in these processes are sparse. In the present work, we show that the atypical RhoGTPase, Rnd3 (also known as RhoE), plays an important role for cortex development during the formation of the brain. RND3 knock-out embryos display cortical defects suggestive of a migration impairment of radial progenitors. Accordingly, overexpression of Rnd3 by in utero electroporation (IUE) induces a stack of the migrating neurons in deep layers while its acute ablation makes the neurons to reach faster the cortical plate. Rnd3 function is regulated by phosphorylation and by farnesylation-mediated membrane tethering. By generating specific Rnd3 mutants and by using IUE, we provide evidence of the role of each of these regulatory mechanisms for Rnd3 function in vivo. Finally, we have explored the function of Rnd3 as a downstream signalling effector of FLRT proteins, which have been involved in different aspects of cortical development. We show that FLRT3 and Rnd3 interact and co-localize in heterologous cells, and we have narrow down the interaction domain in Rnd3 to a stretch of eight aminoacids close to the N-terminus end of the protein. We have modified this interaction domain to generate a Rnd3 mutant (Rnd3F3BM) that is well expressed but is not longer able to bind to FLRT3. This Rnd3F3BM will allow us to explore specifically the role of FLRT3-Rnd3 signalling during cortical development, in vivo.

Role of Lis1 in the Prenatal Development and Migration of Somatostatin-Positive Interneurons

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In this study, we investigated the role of Lis1 in the prenatal development and migration of somatostatin-positive (SST+) interneurons by generating a novel animal model with targeted Lis1 gene deletion in these cells. Previous analyses at postnatal stages revealed a significant reduction in SST+ interneurons in the cingulate cortex and hippocampus of mutant mice, prompting us to study their embryonic development. Lis1 is known to play a key role in neuronal migration during brain development by regulating the movement of the nucleus within migrating neurons, a process known as nucleokinesis. It also participates in the organization of the microtubule network, ensuring proper cell positioning and cortical layering. Embryonic analysis at E14.5 and E16.5 showed fewer migrating SST+ interneurons in the developing cortex, indicating impaired migration. Furthermore, quantitative assessment at E13.5 revealed no co-expression of Cre and Ki67+ (a cell proliferation marker) in the ventricular zone, indicating that the reduction in SST+ interneurons is not due to impaired proliferation. but rather other developmental processes. Notably, a statistically significant reduction in SST+ interneurons was observed in the subventricular, intermediate, and marginal zones at E14.5, as well as in the subplate and marginal zones at E16.5. Additionally, the fractional area occupied by SST+ interneurons in the basal forebrain increased significantly, suggesting possible migration arrest or altered distribution. These findings highlight Lis1's crucial role in SST+ interneuron development, affecting both their migration and positioning. The reduction in SST+ interneurons may contribute to cortical disorganization and excitation/inhibition imbalances observed in Lis1-related pathologies. Further investigations are needed to clarify the mechanisms underlying these disruptions and their long-term functional consequences.

Role of the Gestational Maternal Gut-Microbiota in Regulating the Radial Glia Progenitors of the Hypothalamus and the Medial Extended Amygdala

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The maternal gestational gut-microbiota has emerged as a key regulator of neurodevelopment, with growing evidence linking its alteration to the etiology of neurodevelopmental and neuropsychiatric conditions. Two critical nodes of the social brain, the alar hypothalamus and the medial extended amygdala (EAme), are particularly associated with some of these conditions. Notably, perinatal microbiota changes have been linked with morphological alterations in both brain regions and microbiota-based interventions have been shown to modulate endogenous oxytocin levels in the hypothalamus.
Based on these findings, we hypothesized that the maternal microbiota influences the development of the alar hypothalamus and the EAme. To investigate this, we depleted the maternal gut microbiota in pregnant mice using an antibiotic cocktail and collected embryonic brains at embryonic day E15.5. We then analyzed radial glia progenitors (RG) via immunofluorescence to detect relevant markers, including Nestin or Sox2, in two embryonic domains controlling the specification of both the EAme and alar hypothalamus: the telencephalon-opto-hypothalamic domain (TOH) and the supraopto-paraventricular hypothalamic domain core part (SPVco). Our findings revealed a decreased number of apical RG endfeet in the ventricular zone of both TOH and SPVco in antibiotic treated mice, accompanied by an increased number of basal RG. Interestingly, the total number of apical RG soma in the ventricular zone remained unchanged in both domains. These results suggest that the maternal gestational gut microbiota plays a role in modulating RG structure, production and dynamics, potentially leading to morphological defects in these brain areas. Further research will elucidate its specific role in the differentiation and specification of neuronal populations within these key neurodevelopmental hubs.

Keywords: Microbiota-gut-brain-axis, maternal microbiota, neurodevelopment, medial extended amygdala, hypothalamus, oxytocin

Rptor/mTORC1 Function in Radial Glia Progenitor Cell Lineage Progression

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The cerebral cortex is composed of an extraordinary number of neuronal and glial cell types assembling into cortical circuits that account for cognitive abilities. Remarkable heterogeneity in the spectrum of cortical cell types has been described on the basis of global transcriptome analysis. How cell-type diversity is generated by radial glia progenitor (RGP) cells during development is however still poorly understood. Here we focus on the Rptor/mTORC1 signaling pathway and its role in RGP cell lineage progression and cortical neurogenesis. By using Mosaic Analysis with Double Markers (MADM) technology we ablated Rptor/mTORC1 either sparsely in single clones or in the entire tissue. Such paradigms enabled us to determine the relative contributions of cell-autonomous. Rptor/mTORC1 function and their interaction with global tissue-wide mechanisms. We found that Rptor is not cell-autonomously required for cortical neurogenesis but rather for survival of defined populations of cortical excitatory neurons. Strikingly, RNA sequencing analysis unveiled differential requirement for Rptor function across distinct specific projection neuron classes, implying that mTORC1 signaling fulfills critical roles in the generation of cortical cell-type diversity.

Temporal and spatial regulation of microglial colonization

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Microglia, unlike other brain cells, do not originate from the neuroectodermal layer but instead migrate from the yolk sac at early embryonic stages and subsequently proliferate and colonize the brain parenchyma. However, how this colonization is orchestrated to reach a tessellated distribution remains to be elucidated. To analyze microglia colonization dynamics over time, we developed a mathematical model of differential equations to track microglia densities within different brain layers and fitted it to experimental data using Bayesian (Markov chain Monte-Carlo) inference. The in silico results suggested that microglia in both the hippocampus and cerebellum synchronously turn off their proliferation program by P5, before their final numbers are achieved by P14, implying the existence of a previously unidentified key developmental switch. These results also suggested that microglial proliferation is controlled by mechanisms alternative to contact inhibition, which would be expected to act when microglia reach their final density. Analysis of nearest neighbour distances did not show significant differences across ages for all microglia. However, proliferative microglia were more distant from each other compared to mitotically quiescent cells, suggesting a potential spatial cue in the control of microglial proliferation. To test the role of contact inhibition and understand the colonization dynamics in space, we are developing a second mathematical approach using an agent-based model in which we analyze the local density of proliferative and mitotically quiescent microglia over time. Elucidating the regulators of microglial developmental milestones will provide a better understanding of their role in brain development and neurodevelopmental disorders in which microglia have been involved.

The control of stem cell quiescence in early postnatal dentate gyrus is essential for establishing long-lasting adult hippocampal neurogenesis

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During embryonic and postnatal development of the dentate gyrus (DG), neural stem cells (NSCs) proliferate, migrate and generate mature granule neurons and astrocytes. Unlike other brain regions, NSCs remain in the subgranular zone of the adult DG, mostly in a state of deep quiescence, with the ability to re-enter cell cycle and generate new neurons throughout life. The transitions back and forward from a proliferative state to a temporal shallow quiescent state ensure the maintenance of stem cell population. NSCs in the DG enter in a quiescent state predominantly during the first postnatal week, however, it is unclear when and how adult NSCs acquire different quiescence sub-states (deep or shallow) during postnatal development.
Recently, we have determined that transcription factor Sox5 is required for the activation of adult NSCs and the generation of new neurons. Now, using conditional mouse mutants for Sox5 during embryonic (Sox5Nestin) or early postnatal development (Sox5Sox2-creERT), we describe a critical window around P14 when NSCs build up a shallow or intermediate quiescent state. During that period, an excess in shallow quiescent NSCs leads to aberrantly over activated NSCs and an excess in neurogenesis in Sox5 mutant adult DG. Consequently, mature adult Sox5Nestin mice show a premature reduction of the NSC pool and a decrease in adult neurogenesis. Finally, we have uncovered that a deregulation in the BMP canonical signaling pathway is behind the alterations at P14 in the early establishment of the shallow quiescent NSCs population following Sox5 loss. In conclusion, we have determined that Sox5 is required to limit the size of the shallow quiescent population of NSCs during early DG development that ensures long lasting adult hippocampal neurogenesis.

Transcription Factor Cooperation and Chromatin Accessibility in Neuronal Identity

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The complexity of the nervous system stems from the remarkable diversity of its fundamental units, the neurons. Neurons encompass a large variety of information processing and propagating cells with shared features, as well as highly divergent features including morphology and physiology. The identity of each neuronal type is established by unique transcriptional programs during development and actively maintained throughout life. Disruptions in these programs can lead to severe neurodegenerative and neuropsychiatric disorders, such as Alzheimer's and Parkinson's disease. A crucial step in understanding nervous system function is to define the molecular identity of a fully differentiated neuron. Genetic and biochemical studies have identified a conserved set of neuronally expressed proteins, many of which are involved in synaptic vesicle dynamics that are essential for neuron-neuron communication. The genes encoding these proteins form a common molecular toolkit shared by all neurons and are key to defining neuronal identity. Studies in C. elegans have shown that the expression of these shared synaptic genes is regulated through the combined action of CUT homeobox transcription factors, which are broadly expressed across the nervous system, and neuron type-specific master regulators known as terminal selector (TS) transcription factors. Previous research has shown that the CUT homeodomain binding motif is strongly associated with differential chromatin accessibility between neurons and fibroblasts, suggesting that CUT factors may function as pioneer factors. We propose that CUT factors regulate the binding of additional TFs by modulating chromatin accessibility. To assess the impact of CUT factors on the neuronal chromatin landscape, we have developed a protocol to isolate nuclei from all C. elegans neurons using FACS, followed by profiling their chromatin accessibility in CUT homeobox gene mutants. This work will enhance our understanding of the molecular mechanisms that regulate synaptic gene expression and neuronal function.

Unraveling the sex-specific function of Dmrt5 in the developing midbrain using multi-omics

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The mammalian brain presents sex differences at molecular and structural levels. Gonadal hormones, genes and epigenetics contribute to the generation of these sex differences and might be crucial for the sex bias in mental disorders. Dmrt transcription factors have emerged as conserved regulators of sex-specific traits across metazoans, playing a key role in the sexual differentiation of the nervous system in invertebrates. However, it is unknown whether Dmrts regulate the sexual differentiation of the mammalian brain.

We performed genetic analyses across 10 psychiatric phenotypes. We found an association between DMRTA2 (DMRT5) and attention deficit/hyperactivity disorder (ADHD), a condition that shows higher prevalence among the male population and evidence for alterations in the cortex and midbrain. In mice, Dmrt5 is well characterized in the control of cortex development, and in humans, a mutation in DMRTA2 leads to microcephaly and lissencephaly. This study aims to unravel the sex-specific function of Dmrt5 in the mouse midbrain.

We showed that Dmrt5 null mutant mice present patterning defects within the midbrain at early developmental stages in both sexes, evidenced by the miss-expression of transcription factors such as Barhl1, Engrailed1 or Foxa1. Moreover, in late developmental embryos, coinciding with the embryonic testosterone release, we observed that Dmrt5 suppresses transcriptional differences between the sexes. By using multi-omics, including single-cell RNA sequencing, we found a male-specific subpopulation in Dmrt5 mutants. Interestingly, the human orthologous genes dysregulated in Dmrt5 mutant males (N=166) were associated to ADHD in a gene-set analysis (P=0.004) using genome-wide association studies (38,691ADHD cases; 186,843 controls). The mis-regulation of these gene networks might predispose for ADHD with a stronger prevalence in the male population. In conclusion, Dmrt5 emerges as a crucial transcription factor required for the accurate development of the midbrain and for achieving a transcriptome equivalence between male and female midbrains.

Unraveling the Vertebrate Bauplan across Development: Bridging Hypothalamic and Prethalamic Territories

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The current prosomeric model identifies the diencephalon as the caudal part of the prosencephalon, consisting of three prosomers p3 to p1 (prethalamus, thalamus and pretectum, respectively). The prethalamic region is the most rostral prosomere, delimited caudally by the zona limitans intrathalamica (zli), which separates it from the thalamus, and rostrally by the hypothalamus, which belongs to the secondary prosencephalon. However, studies in amphioxus suggest a possible closer ontological relationship with the rostral telencephalic-hypothalamic territories than with the other diencephalic prosomers, based on the expression of several key developmental genes. The specification of these regions is precisely regulated by molecular signals that influence the progenitor domains in the emergence of differentiated derivatives. Thus, the prethalamic region plays a crucial role in the development of forebrain structures as a key signalling center for molecular information from the zli and the basal plate. Hence, this region and its evolutionary variations play an essential role in neural patterning and regionalization, constituting a pivotal point in the phylogenetic diversification of prosencephalic structures in vertebrates. Our genoarchitectural study in the Xenopus laevis brain at early embryonic stages, just before the formation of the zli, shows a closer relationship of the p3 territory with the hypothalamo-telencephalic region. Therefore, the ontological relationship of the prethalamic region is likely closer to the hypothalamic and subpallial territories, which could imply the existence of a common territory of origin during development, not only in primitive chordates, but also in anamniote tetrapods, and possibly in all vertebrates.

Untangling the cis-regulatory landscape controlling TBR2 expression in the developing human cortex: of enhancers, TADs and lncRNAs

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Embryonic cortex development relies on the combined activity of transcription factors and signaling molecules displaying complex expression patterns. Structural variants that disrupt the regulatory landscape of these developmental genes are often associated with severe brain abnormalities.

TBR2 is a transcription factor crucial for mammalian cortex development, since it controls the proliferation and differentiation balance of intermediate progenitors, a population of fast amplifying progenitors which importantly contribute to production of glutamatergic neurons. TBR2 is located in a gene-poor genomic region, in which, epigenetic profiling data suggest the presence of numerous cis-regulatory elements. A genomic translocation disrupting the gene desert portion on the 3' side of the TBR2 locus is associated with severe brain alterations (microcephaly, polymicrogyria, etc.)

Here, we combine the use of human cortical organoids with CRISPR genome editing and recombination-mediated transgenesis in iPSC to characterize the regulatory landscape of the human TBR2 locus and comprehend how its disruption could affect cortex development. First, we assessed chromatin accessibility by ATAC-seq in our CO cultures and TBR2-enhancer contacts by 4C-seq. We confirmed that our candidate sequences display dynamic accessibility changes, and that the TBR2 promoter is contacted by elements of both gene deserts. Interestingly, these interaction domains are organized in two Topologically Associated Domains (TADs) with TBR2 sitting at their border. Deletion of each of these two regulatory domains impact TBR2 expression in cortical neural precursors. Besides, we have identified at least two non-coding RNAs, METEOR and LACUNA, located within the TBR2 regulatory domains. These RNA genes are overexpressed after the neurogenesis onset and seem to share regulatory elements with TBR2. Our findings hint a potential role of these RNA genes in the post-transcriptional control of TBR2 activity and in the establishment of TBR2 domain boundaries.

Unveiling the dynamic link between proliferation and phagocytic efficiency in brain development: the role of IKAROS

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Microglia are the specialized phagocytes of the brain, and they play a key role in maintaining its homeostasis. They derive from yolk sac progenitors that invade the brain parenchyma at embryonic stages. However, the spatiotemporal dynamics and molecular mechanisms by which microglia acquire their functionality are largely unknow. Here we show that microglia are not born-efficient phagocytes. Using confocal imaging to visualize mice brain slides from P2 to P60, we found that microglia colonized the brain in the first two postnatal weeks and achieved final number by P28 in the hippocampus and P21 in the cerebellum. This increase was driven by highly proliferative cells during the first postnatal week, which progressively became quiescent. We next studied the relationship between microglial colonization and phagocytosis and found that its efficiency increased progressively in postnatal mice until P21 whereas developing microglia were not able to respond to apoptotic challenges until P14. These findings showed microglial phagocytic efficiency increased after they colonized the brain. Microglial proliferation and phagocytosis efficiency were negatively correlated, suggesting that they are exclusive processes. To test whether colonization was required for phagocytosis maturation, we impaired microglial proliferation using both an IL34 KO mice model and pharmacologically inhibiting CSF1R, founding that phagocytic efficiency was reduced. We are further exploring the functional effects of impairing microglial proliferation during development using a mouse model deficient in IKAROS, an inhibitor of KDM5B which regulates proliferation. Our preliminary data shows that IKAROS KO mice have reduced microglial proliferation and density, resulting in impaired phagocytosis. These results underscore the tight link between proliferation and phagocytosis and highlight the fact that microglia need to proliferate and colonize the brain before acquiring full phagocytic efficiency. This is highly relevant as microglia are long-lived cells and early impairments on their maturation may have an impact in the long term.

UNVEILING THE IMPACT OF CHROMATIN REMODELER CHD2 DEPLETION IN HUMAN CORTEX DEVELOPMENT

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The diversity of neurons composing the human cerebral cortex arise during embryonic development through a process called neurogenesis. Epigenetic modifications play an essential role in its regulation by controlling the activity of neurodevelopmental genes through different and interrelated mechanisms.

CHD2 is a chromatin remodeler whose mutations in humans have been associated with different brain disorders/malformations, including epileptic encephalopathies, autism, cognitive impairment, microcephaly, etc. While Chd2 inactivation in mice alters cortical neurogenesis, it fails to fully replicate the spectrum of clinical phenotypes observed in patients. Moreover, the precise genetic and epigenetic mechanisms through which CHD2 regulates cortical development remain largely unexplored.

We observed that heterozygous CHD2 mutations disrupting its chromatin remodeling activity but sparing the chromatin binding domains correlate with increased severity of pathological phenotypes compared to mutations affecting all its functional domains.

Besides, the human CHD2 locus is predicted to encode transcriptional isoforms not described in mice, which may account for the partial recapitulation of CHD2 pathological phenotypes in mouse models. These human-specific isoforms encode a truncated CHD2 variant, retaining the chromatin-binding domain but lacking chromatin remodeling activity. Consequently, an imbalance in isoform activity due to mutations specifically disrupting the full-length CHD2 variation may contribute to the phenotypic variability observed in patients.

To delve deeper into these mechanisms, we characterized the human CHD2 isoform expression in iPSC- derived cortical organoids, which faithfully recapitulate the cortical neurogenic process. Furthermore, utilizing CRISPR/Cas9 genome editing, we generated iPSC lines harboring several CHD2 mutations, mimicking those found in patients. Subsequent analysis of cortical organoids derived from these mutant iPSC lines unveiled their impact on organoid morphology and neural precursor populations, shedding light on the intricate role of CHD2 in cortical development and disease pathology. We also compared the transcriptional profiles of WT and mutant organoids performing bulk and scRNAseq experiments

NEURONAL EXCITABILITY, SYNAPSES AND GLIA: CELLULAR MECHANISMS

Analysis of synaptogenic processes in a selective neurodegeneration model, the mutant mouse Purkinje Cell Degeneration

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The cerebellum coordinates body movement, requiring stable and functional neuronal networks. Microglia is essential in synapses establishment and remodeling. This study analyses the PCD mutant mouse model, which undergoes rapid Purkinje cell degeneration and exacerbated microgliosis. The recipient group has investigated the administration of the microglia inhibitor PLX5622 following a schedule of 4 doses from P15 to P24. It improves Purkinje neurons survival in PCD mice. However, it is unknown if PLX5622 affects other microglia´s functions, such as synaptogenesis and synaptic pruning; or the effect of the degenerative process on those functions themselves.
Immunohistochemistry employing antibodies against Calbindin D28K, Iba1 and Psd-95 was used to label Purkinje cells, microglia and synaptic puncta respectively. Colocalization between the different labelings was evaluated to analyze the area of Purkinje cells and puncta monitored by microglia and the area of Purkinje cells that was occupied by puncta. Two stages were analyzed: pre-degenerative (P15) and peak degeneration (P25). At P15, the effect of the mutation was evaluated before Purkinje cell death. At P25, WT and PCD animals, with and without PLX5622, were compared to study its impact on synapses and neurodegeneration.
We found that microglial inhibition affects synaptic maturation in the absence of a pathological environment. Likewise, microglia overactivated by neuronal damage interferes with synaptic establishment even before the degeneration starts, presenting an apparent compensatory role. PCD mouse microglia that survives the treatment is positioned over the synaptic connections, possibly with a neuroprotective function. Ongoing analysis aim to explain how much of the surviving microglia´s area is employed in the maintenance of these synaptic contacts in a degenerative environment. These are focused on the perspective of the microglia. Unlike previous studies, which were focused on the perspective on Purkinje cells and their synaptic puncta. This work highlights the important microglial function, especially in neurodegenerative diseases.

Anatomical evidence of hemichannel-mediated communication between microglia and neural precursor cells of the subventricular zone

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Neural progenitor cells (NPCs) of the adult mammal subventricular zone (SVZ) generate neuroblasts that migrate to the olfactory bulb, where they differentiate into mature neurons. Microglial cells in the neurogenic niche of the SVZ intervene in the control of neurogenesis through the release of soluble factors and extracellular vesicles. Other communication pathways between microglia and NPCs, such as gap junctions or hemichannel-mediated release of small molecules, might also contribute to the modulation of SVZ neurogenesis. In this study we explored by immunohistochemistry the expression of the hemichannel proteins connexin43 (Cx43) and pannexin1 (Panx1) in microglia and NPCs of the adult rodent SVZ. Our results show that SVZ NPCs and microglia express both Cx43 and Panx1, the latter in a higher proportion. In addition, 13.4 ± 0.7% of microglial cell perimeter establishes direct contact with SVZ NPCs. Cx43 and Panx1 are occasionally found in the interface of microglia-NPC direct contact zones, suggesting that gap junctions might be formed between these two cell types in the SVZ. Electron microscopy revealed the presence of gap junctions in SVZ microglial cells, albeit rarely. In summary, our results provide an anatomical basis for a role of gap junctional and hemichannel-mediated communication between microglial cells and NPCs in the regulation of SVZ neurogenesis.

Acknowledgements: EU-Ministerio de Hacienda y Función Pública-European Funds-Junta de Andalucía for RGB predoctoral contract.

Astrocyte metabolic support to adaptive myelin plasticity following sensory deprivation of the somatosensory cortex

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Recent studies show that horizontal L2/3 axonal projections exhibit increased elongation of myelin segments following monocular deprivation. Such adaptive myelin plasticity may improve the synaptic efficacy of corticocortical connections, potentially playing a role in mediating the reorganization of cortical maps after sensory deprivation. Here, we explored the relationship between axonal myelination and the level of cortical reorganization in distinct layers of the somatosensory cortex following central sensory deprivation. The complete thoracic spinal cord was used to induce sensory deprivation in cortical areas receiving information from the hindlimbs. Sensory-evoked potentials in response to contralateral forelimb stimulation was performed to determine the strength of the synaptic connectivity between the deprived and intact cortex in injured and control animals. Our results show that sensory deprivation enhanced L2/3 corticocortical connectivity, observed as an increased magnitude and slope of the deprived neurons' responses.
Next, we explored whether the increased L2/3 synaptic efficacy was associated with myelin remodeling. While the number of Olig2+ cells, Ranvier nodes, and mature myelinating oligodendrocytes were not affected, MBP staining and internodal distance were increased, suggesting a process of adaptive myelin plasticity from existing segments. Furthermore, this myelin plasticity was specific to parvalbumin-inhibitory neurons, as observed by measurements of myelin segments in Cre-lines for VIP, PV, and Stt neurons. Given that astrocytes provide metabolic support for oligodendrocyte myelination, we used IP3R2 knockout mice to determine the role of these cells in adaptive myelin plasticity. Sensory-deprived IP3R2-/- mice did not show changes in sensory-evoked potentials or myelination. Additionally, deprived IP3R2 astrocytes presented an increased number of intracellular glycogen granules, suggesting deficits in lactate production to support oligodendrocyte myelination. Overall, our data indicate that astrocytes contribute to L2/3 adaptive myelin plasticity during cortical reorganization following sensory deprivation by facilitating metabolic support to oligodendrocytes.

Astrocyte Modulation of Regional Neuronal Network Development

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Precise development of neural networks relies upon intricate organization within and across brain regions. In this framework, astrocytes play pivotal roles orchestrating formation of nascent circuitry. Through direct contact with neurons and secretion of signaling molecules, astrocytes engulf synapses, mediate plasticity, and contribute to neurogenesis. Furthermore, as astrocytes have been shown to induce the formation and stabilization of synaptic contacts, they have also been hypothesized to be responsible for specification of the synapse. Our group has previously highlighted the transcriptional differences between astrocytes and neurons across brain areas. Building upon these findings, we hypothesized that region-specific transcriptomic profiles underpin distinct activity patterns within developing networks. We discovered intrinsic properties defined by brain region in astrocytes are sufficient to modulate developing neuronal networks in cocultures comprised of neurons and astrocytes from diverse brain regions. Notably, calcium activity in matching cocultures from the thalamus exhibits inherent differences compared to those from the cortex. However, this distinctive phenotype changes when astrocytes and neurons from both regions are mismatched, showing a bias towards the region from which the astrocytes originated. Additionally, we observed region-specific phenotypes in cell morphology and expression of specific synaptic markers are reliant on the specific brain region from which the astrocytes are isolated. Lastly, we determined that these phenotypes are reproducible using conditioned media from regionalized astrocytes. Further investigation will elucidate underlying mechanisms responsible for regional specialization of neural networks, ultimately highlighting an emerging role of astrocytic contribution to brain region specification.

Astrocytic GLUT1 Ablation Modulates Ca^{2+} Dynamics and Restores Neuronal Activity in Alzheimer's Disease

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Glucose availability in the CNS is critical for neuronal function, and glucose levels in the brain regulate neuronal activity. Regulation of blood glucose supply to and within the brain is controlled via glucose transporter GLUT1 that is highly expressed in astrocytes. It is known that Alzheimer's disease (AD) patients feature decreased GLUT1 levels, and that GLUT1 ablation in brain vascular endothelial cells accelerates AD progression. Nonetheless, the possibility that astrocytic GLUT1 plays a functional role in AD pathology has never been studied.

Preliminary data obtained by our group has shown that, unexpectedly, GLUT1 ablation at early stages of AD (at 2 months of age) induced an improved cognitive performance at advance stages of the disease (at 8 months of age). This cognitive improvement appears to be related to a higher baseline Ca2+ event probability and duration of GLUT1 depleted astrocytes observed in ex vivo hippocampal slices. In the present study, using the fiber photometry technique, we have demonstrated that the improvement in astrocytic Ca2+ dynamics observed in slices also occur in vivo in hippocampal CA1.

AD mouse models reveal early-stage hyperexcitability and seizures in the hippocampus, while late stages show hypoactivity, indicating a shift in the excitatory:inhibitory balance over disease progression. To assess astrocytic GLUT1 ablation's impact on hippocampal circuits, we used in vivo electrophysiology in anesthetized AD GLUT1 depleted mice. Noteworthy, we found that 8-month-old mice with GLUT1 deletion at 2 months restored neuronal hypoactivity seen in late-stage AD, probably linked to the improved Ca2+ and with consequent behavior improvement.

In the light of the emerging cross talk between energy metabolism and AD, the glucose transport capacity of astrocytes may be a key factor. In this line, the present study provides a better understanding of mechanisms underlying AD and new directions for clinical research for better therapy development.

Astrocytic insulin receptor ablation precipitates Alzheimer's disease onset

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Insulin is a key regulator of glucose homeostasis and metabolism, but its role in the central nervous system remains incompletely understood. While insulin signaling in neurons has been widely studied, its function in astrocytes is still unclear. Astrocytes play a fundamental role in maintaining brain homeostasis by providing metabolic and structural support to neurons, regulating synaptic activity, and contributing to the blood-brain barrier. Disrupting insulin signaling in astrocytes could compromise these essential functions, ultimately altering neuronal activity and leading to cognitive deficiencies. Emerging evidence posits brain insulin resistance as a critical factor in Alzheimer's disease (AD), yet the specific contribution of astrocytic insulin signaling to AD pathophysiology is largely unexplored. In this scenario, our study aims to evaluate the impact of astrocytic insulin receptor (IR) deletion on AD.

Our findings indicate that astrocytic IR depletion increases mortality rates and accelerates AD pathology in APP/PS1 mice, leading to cognitive impairments as early as 4 months of age. Notably, IRΔGFAP mice exhibit astrocyte morphological alterations distinct from the typical reactive astrogliosis observed in AD. However, no significant changes were detected in the amyloid pathology (assessed via ELISA, FACS, and immunofluorescence) or Tau levels (quantified by Western Blot). Furthermore, ex vivo Ca^{2+} imaging in hippocampal slices revealed aberrant astrocytic Ca^{2+} dynamics in IRΔGFAP mice, characterized by increased frequency of short Ca^{2+} transients that persist even after neuronal activity inhibition. Consistently, in vivo electrophysiological recordings in the CA1 hippocampal region demonstrated neuronal hyperexcitability, further supporting the notion that astrocytic IR depletion disrupts astrocyte-neuron communication and potentially exacerbates the AD-related network dysfunction.

These results highlight astrocytic IR as a potential therapeutic target for AD, offering a novel approach beyond classical pathological markers. Understanding its role in brain energy homeostasis could lead to more effective strategies for this devastating disease which undeniably requires innovative solutions.

BENEFICIAL EFFECTS OF ASTROCYTIC GLUT1 ABLATION IN ALZHEIMER'S DISEASE

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Aberrant brain bioenergetics is proposed as one of the underlying mechanisms in Alzheimer's disease (AD) pathology. Indeed, a prominent drop in brain glucose uptake is observed prior to the manifestation of AD symptoms. Glucose is the main energy source of the brain, and its supply from the blood to the brain is controlled by the glucose transporter GLUT1, highly present in astrocytes. Thus, astrocytes are located at the interface between vessels and neurons, putting them in a privileged position to control brain glucose uptake. In the present work, we hypothesized that astrocyte-specific GLUT1 deletion could accelerate the onset of AD.

To explore the role of astrocytic GLUT1 in AD, we generated an astrocytic GLUT1 knockout (KO) mouse model at 2 months of age and assessed its effects at 8 months. Surprisingly, astrocytic GLUT1 deletion decreased the elevated mortality of APP/PS1 mice. Moreover, astrocytic GLUT1 ablation rescued the cognitive impairments shown by APP/PS1 mice in the Morris water maze and fear conditioning memory tests. These effects were unrelated to Aβ pathology, as both amyloid plaques and Aβ levels were unchanged. Interestingly, our data showed a higher baseline Ca2+ event probability and duration of GLUT1 depleted astrocytes, indicating elevated Ca2+ activity levels. Noteworthy, chemogenetic perturbation of astrocytic Ca2+ activity totally abrogated the cognitive improvement elicited by astrocytic GLUT1 ablation.

In conclusion, GLUT1-ablated astrocytes maintain the ability to metabolically cope with Aβ challenge, and far from worsening AD pathology, astrocyte-specific GLUT1 ablation prevents both the increased mortality and impaired cognition exhibited by APP/PS1 mice.

Bidirectional communication between medial prefrontal cortex and accumbal astrocytic ensembles

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Astrocytes, long considered a uniform cell type, have increasingly been recognized over the past decade for their critical roles in brain function. Important efforts are underway to elucidate how different astrocyte populations interweave in brain circuits. The nucleus accumbens (NAc), a central hub in the reward system, integrates inputs from multiple afferents that converge in medium spiny projection neurons expressing D1 or D2 dopamine receptors. In particular, inputs from the medial prefrontal cortex (mPFC) have been implicated in action-outcome associations and cognitive flexibility in the context of goal-directed behavior. However, the role of NAc astrocytes in this pathway and their interaction with local circuits remains poorly understood, partly due to the neglect of their functional specialization. Using a combination of electrophysiology and live imaging in acute slices, we studied the plasticity of mPFC-NAc synapses and how it differs in D1 and D2 neurons, focusing on the contribution of the astrocytic ensemble activated by the mPFC. We characterized the spatial distribution of this ensemble in the NAc, its interactions with mPFC afferents, and the consequences of its manipulation on synaptic plasticity. In addition, we show that specific astrocytic ensembles in the NAc exhibit preferential responses to either D1 or D2 neurons, highlighting the functional specialization of astrocytes within the NAc. These results illustrate how different subsets of astrocytes respond to local circuit activity. In the future, goal-directed behavioral tests will be employed to study the behavioral output of these astrocytic ensembles, as well as their local and long-range effect on NAc and mPFC neuronal activity in vivo.

Bioelectric Signals in the Gut-Brain Connection: How Bacteria Influence Neuronal Activity

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Bioelectric signaling-encompassing ion flows, voltage gradients, and electric fields-is fundamental not only for excitable cells, such as neurons, but also for organism-wide processes such as development, regeneration, and cancer. Recent evidence extends the role of bioelectricity beyond eukaryotic systems, revealing its importance in bacterial physiology and gut microbiome regulation. While it is clear that bacteria in the gut and neurons in brain communicate (the microbiota-brain-gut-axis), most of the fundamental questions in this field are wide-open. Crucially, the community and state-of-the-art work focuses almost exclusively on molecular (hormone receptors, immune cell signaling, etc.) and not – as we propose to do – on top-down, information-based approaches, which are needed to fully understand the high-level meaning behind the mechanisms.

In this study, we investigated the influence of bacteria on neural bioelectric properties by analyzing transcriptomic responses following short-term direct interactions between neurons and the probiotic bacterium Lactiplantibacillus plantarum. We further examined neuronal viability, synaptic plasticity, and bioelectric profiles across different bacterial species, interaction durations, and concentrations, comparing probiotic and opportunistic strains. Our results demonstrate that neurons detect bacterial presence in the culture medium, triggering significant transcriptomic changes related to bioelectricity, excitability, and synaptic plasticity. These responses are species-specific and modulated by interaction conditions.

Our results suggest that bioelectricity plays a key role in neuron-bacteria interactions, reveling an underexplored interkingdom communication network. Understanding how the gut microbiome influences neuronal function and identifying bacterial species and conditions that promote beneficial neural effects could open new avenues for targeted interventions and therapeutic strategies for nervous system disorders.

Bridges in movement: Deciphering the Dynamism of the Synaptic Cleft

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Precise transmission of information at synapses is crucial for sustaining cognitive functions, and its disruption can lead to neurological and psychiatric disorders such as schizophrenia, epilepsy, or autism. Despite decades of research, a comprehensive understanding of presynaptic trafficking in neurotransmission remains elusive. Developing a comprehensive picture of the trafficking proteome of firing synapses would provide a complete molecular view of synaptic biology allowing to unbiasedly identify previously-unannotated synaptic proteins whose trafficking on demand is essential to support neurotransmission. To tackle this issue, we combine HRP-based proximity labelling in synaptic clefts, proteomics and genetic tools in primary cortical neurons to study the activity-driven synaptic exocytic proteome. Our results show that neuronal activity triggers the exposure of not only synaptic vesicle and dense core vesicle proteins, but also other non-canonical proteins, such as trans-synaptic proteins. These proteins, which are essential in neurotransmission by forming trans-synaptic connections, appear not to be localized in synaptic vesicles as they exhibit unconventional exocytosis events in firing synapses. These findings raise a fundamental question: What are the molecular characteristics and functions of non-standard exocytic organelles, and could understanding them uncover new mechanisms for regulating synaptic function?

Decoding THC-related impairments: the role of astrocytic ensembles in the Nucleus Accumbens

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Cannabis is the most commonly used illicit drug among adolescents. Although it is often perceived as harmless, emerging evidence shows that it impairs spatial learning—a process that primarily depends on the coordinated activity between the ventral hippocampus (vHip) and the Nucleus Accumbens (NAc). The NAc integrates motor and limbic information from various glutamatergic inputs, such as medial prefrontal cortex, basolateral amygdala, and vHip. Regarding this, our group has previously identified pathway-specific neuron-astrocyte networks in the NAc. Moreover, astrocytic CB1Rs have been shown to modulate synaptic transmission and long-distance communication between neuronal populations. Here, our goal is to investigate the role of astrocytic ensembles in the NAc in impairments related to the use of tetrahydrocannabinol (THC), the psychoactive component of cannabis.

Using fiber photometry, we analyzed astrocytic calcium and glutamate dynamics in NAc after chronic administration of 1mg/kg THC in wild-type and p38αMAPK-/- (Astrop38α) mice. We also conducted electrophysiological experiments to examine synaptic plasticity. Furthermore, the Barnes Maze test was employed to evaluate the effects of THC on spatial learning. Finally, using a combination of AstroLight, a new tool developed in our group, and optogenetics, we identified and selectively manipulated the astrocytic ensemble within the NAc involved in vHip–NAc circuit to analyze its behavioral implications.

We observed: (1) THC increases astrocytic calcium activity and glutamatergic tone within the NAc; (2) the THC-induced glutamatergic alterations are absent in Astrop38α mice, implicating p38αMAPK signaling in astrocyte-mediated plasticity; (3) astrocytic glutamate release within the vHip->NAc ensemble is required for THC-induced cognitive deficits; and (4) targeted attenuation of THC-induced calcium activity in this ensemble prevents both spatial learning and synaptic plasticity impairments.

These results highlight a critical role for astrocytic ensembles in shaping behavior and emphasize their potential as therapeutic targets for mitigating the cognitive consequences of THC exposure.

Developmental Spike Timing-Dependent Long-Term Depression Requires Astrocyte d-Serine at L2/3-L2/3 Synapses of the Mouse Somatosensory Cortex

Developmental Spike Timing-Dependent Long-Term Depression Requires Astrocyte d-Serine at L2/3-L2/3 Synapses of the Mouse Somatosensory Cortex

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Spike timing-dependent plasticity (STDP) is a learning rule important for synaptic refinement and for learning and memory during development. While different forms of presynaptic t-LTD have been deeply investigated, little is known about the mechanisms of somatosensory cortex postsynaptic t-LTD. In the present work, we investigated the requirements and mechanisms for the induction of developmental spike timing-dependent long-term depression (t-LTD) at L2/3-L2/3 synapses in the juvenile mouse somatosensory cortex. We found that postnatal day (P) 13-21 mice of either sex show t-LTD at L2/3-L2/3 synapses induced by pairing single presynaptic activity with single postsynaptic action potentials at low stimulation frequency (0.2 Hz) that is expressed postsynaptically and requires the activation of ionotropic postsynaptic NMDA-type glutamate receptors containing GluN2B subunits. In addition, it requires postsynaptic Ca2+, Ca2+ release from internal stores, calcineurin, postsynaptic endocannabinoid synthesis, activation of CB1 receptors, and astrocytic signalling to release the gliotransmitter d-serine to activate postsynaptic NMDARs to induce t-LTD. These results show direct evidence of the mechanism involved in developmental postsynaptic t-LTD at L2/3-L2/3 synapses, revealing a central role of astrocytes and their release of d-serine in its induction.

Different Ras isoforms regulate synaptic plasticity in opposite directions

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Learning and memory are fundamental cognitive functions that rely on the brain's ability to adjust neuronal connections in response to experience, a process known as synaptic plasticity. Although extensive research has focused on this phenomenon, the molecular mechanisms underlying long-term potentiation (LTP) and long-term depression (LTD) are not yet fully understood.

Ras, a small GTPase, functions as a molecular switch regulating intracellular signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway. This pathway has been implicated in synaptic plasticity, particularly in LTP and metabotropic glutamate receptor-dependent LTD (mGluR-LTD). However, while Ras has been established as necessary for LTP, its role in mGluR-LTD remains unclear. Additionally, mutations in genes controlling the Ras/MAPK pathway contribute to neurodevelopmental disorders known as RASopathies. Interestingly, despite the high sequence conservation among Ras paralogs (commonly referred as isoforms), H-Ras, K-Ras and N-Ras, mutations in each lead to distinct syndromes, suggesting functional differences.

In this work we aimed to investigate Ras involvement in mGluR-LTD and examine whether different isoforms perform unique or overlapping roles in synaptic plasticity. Using genetically modified animal models, biochemical assays, electrophysiological recordings, and behavioral analyses, we demonstrate that H-Ras, the predominant isoform in the brain, is not required for LTP but is contributing to mGluR-LTD. H-Ras activation occurs in dendritic spines, relying on upstream c-Src activity and inducing MAPK signaling and protein synthesis. Its deletion impairs object recognition, spatial, and social memory. Conversely, K-Ras is critical for LTP but not for mGluR-LTD.

These findings reveal functional specificity between H-Ras and K-Ras, which aligns with their distinct subcellular distributions, also described here. By providing molecular insights into Ras signaling in synaptic plasticity, this study advances our understanding of RASopathies and fundamental neurobiological processes.

Differential effects of tDCS on cortical excitatory and inhibitory neurons in awake mice: an optogenetic study

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Transcranial direct-current stimulation (tDCS) modulates neural activity by inducing polarity-dependent changes in cortical excitability. A key aspect of its effects is the regulation of the excitation-inhibition (E/I) balance, crucial for neural network function. Here, we investigate how tDCS influences excitatory and inhibitory neurons in the awake mouse using optogenetics.
C57J mice (n=40) were injected with adeno-associated viruses (AAVs) carrying the gene for the light-activated channel ChR2 under cell-type-specific promoters. Twenty mice received AAV-CaMKII-ChR2(E123A)-mCherry to target glutamatergic neurons, while another twenty received AAV-mDlx-ChR2(E123A)-mCherry to target GABAergic neurons. After three weeks, a reference electrode was implanted in the left primary somatosensory cortex (S1) for neuronal recordings, an active tDCS electrode was placed over the right S1, and a headplate was fixed to allow intracranial recordings under head-restrained conditions. Electrophysiological recordings of local field potentials (LFPs) and single-neuron activity in response to photostimulation were conducted using optrodes, optical fibers, and the high-density Neuropixels recording system. Neuronal responses were assessed before, during, and after brief tDCS pulses (5 s) at different intensities (±50, ±100, ±150, and ±200 µA) and polarities (anodal and cathodal), both with and without photostimulation.
Interestingly, anodal tDCS increased the slope of LFP responses induced by glutamatergic photostimulation, whereas cathodal tDCS decreased it. In contrast, no significant changes were observed in the LFP slope associated with GABAergic photostimulation. This was further confirmed by firing rate analysis of optogenetically identified neurons, which revealed that glutamatergic neurons exhibited a statistically significant modulation dependent on tDCS intensity and polarity, while no significant modulation was detected in the firing rate of optogenetically identified GABAergic neurons.
These findings suggest that the immediate modulatory effects of tDCS on the somatosensory cortex are primarily mediated by glutamatergic neurons. To our knowledge, this is the first study to characterize tDCS effects at the level of optogenetically identified neuronal populations.

Differential Impacts of Acute, Chronic, and Social Defeat Stress on Microglial Morphology in the Amygdala and Habenula

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Effective stress management is vital for emotional and psychological well-being, as maladaptive responses can lead to the development of various mental health disorders, including depression. This study investigates the effect of different types of stress acute, chronic, and social defeat on microglial morphology in the amygdala and habenula, key regions involved in stress processing and emotional regulation. Specifically, the basolateral amygdala (BLA), central amygdala (CeA), lateral habenula (LHb), and medial habenula (MHb) were examined in rodent models exposed to chronic unpredictable intermittent stress, acute stress (as a PTSD model), and a 10-day SDS stress model in C57BL/6J animals. Microglial morphology was analyzed using anti-Iba-1 immunohistochemistry, and image analysis was performed with FIJI (ImageJ) to quantify morphological changes, inferring activation degree from these alterations.

The findings revealed region-specific microglial alterations in response to distinct stress conditions. Chronic stress in the BLA and CeA led to significant increases in area, perimeter, and Feret diameter, suggesting microglial enlargement linked to stress response mechanisms. In contrast, acute stress caused a decrease in microglial density in the LHb, indicating changes in the stress regulation pathway. Social defeat stress (SDS) induced significant morphological alterations in the BLA and CeA, with increased area, perimeter, and Feret diameter, and reductions in circularity and solidity, reflecting pronounced microglial activation.

These results underscore the differential roles of the amygdala and habenula under varying stress conditions. The amygdala's microglial morphology was particularly responsive to chronic stress, while the LHb microglia showed distinct changes under acute stress. Further research is required to explore the mechanisms linking these microglial changes to stress-related psychiatric disorders. Understanding these differential microglial responses could lead to targeted treatments for conditions such as PTSD, depression, and anxiety.

Funding: PID2020-117463RB-I00 and PID2023-151537OB-I00 funded by MCIN/AEI/ 10.13039/501100011033, P20-00460, UMA20-FEDERJA-112 and the II research-transfer-dissemination plan of the University of Malaga.

Discrete Functional Effects of Epileptiform Discharges on Different Hippocampal Cell Populations

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Epileptiform activity (EA) refers to abnormal excessive neuronal discharges detected by electroencephalographic recordings (EEG) in epileptic patients, where interictal EA is found distributed between seizure episodes. Leveraging a pure model in which EA is induced without causing seizures, we described the chronic presence of epileptiform discharges in hippocampal network activity. However, whether this altered network activity is associated with cellular and synaptic changes in different hippocampal areas remains unclear.

Here we investigate the short- and long-term effects of EA on the cellular and synaptic activity of CA1 pyramidal cells and short-term effect on granule cells of the hippocampus.

In the short-term (i.e., 7 to 15 days after EA induction) we observed hyperpolarization of the resting membrane potential, but no alterations in inwardly rectifying potassium (Kir) channel activity, of granule cells and CA1 pyramidal cells. Although firing properties remained generally unaltered by EA in both populations, action potential afterhyperpolarization was greater in CA1 pyramidal cells, probably reflecting a greater BK channel activity. Synaptic activity of granule cells was not altered by EA, but CA1 pyramidal cells showed greater amplitudes of excitatory postsynaptic currents, suggesting postsynaptic effects of EA.

In the long-term (i.e., 50 to 70 days after EA induction) CA1 pyramidal cells showed distinct cellular alterations compared to the short-term. Membrane resistance was increased and Kir channel conductance was decreased, leading to increased excitability. However, firing properties, synaptic excitation and inhibition and excitatory-inhibitory balance remained unaltered after EA induction. Currently, we exploring the long-term EA effects on granule cells and short and long-term effects on GABAergic cells of the CA1 area.

We conclude that hippocampal EA increases cellular excitability by affecting resting membrane properties and diverse potassium channels rather than firing properties. The mechanisms involved in these changes, however, are distinct for CA1 pyramidal cells in the short and long-term.

Disrupted K+ channels function in Shank3 deficient neurons: autonomic neuronal imbalances and cardiac implications

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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental alterations that arise from a combination of multiple environmental and genetic factors. Recent evidence strongly associates Shank3 protein mutations with ASD-related alterations, and comorbidities implicating excitability imbalances point to a connection between K+ channels and excitability irregularities in Shank3-related ASD. Regarding cardiac abnormalities and although they have only been superficially studied in ASD cases, small alterations in cardiac structure and function have been identified in a Shank3 mouse model of autism. An imbalance between sympathetic and parasympathetic cardiac neuronal activity can lead to autonomic cardiac dysfunction, potentially caused by K+ channel alterations. While the relation between K+ channels and SHANK3 mutations has been explored regarding brain circuitry, little attention has been paid to disruptions in the autonomic nervous system.

For all this, we aimed to identify potential differences in autonomic neuronal excitability in Shank3 mutant mice concerning autonomic heart control and to molecular and electrophysiologically characterize the involved ion channels. We employed perforated patch (whole-cell) technique to compare intrinsic electrical properties of control and Shank3-deficient neurons in the intracardiac ganglia, the nodose (sensory) and the superior cervical ganglion (motor). We also examined different ion channels responsible of the resting potential maintenance (HCN, KCNQ, K2P, A type and delaying rectifiers) that could contribute to the cell excitability imbalances found in Shank3 deficient neurons.

Our findings reveal that Shank3-deficient neurons exhibit a more depolarized membrane potential and enhanced excitability, driven in part by altered TREK, M, and H currents. These disturbances in autonomic neuronal excitability correlated with in vivo measurements of aberrant basal heart rate. Overall, our results suggest that Shank3 mutations may cause K+ channel perturbations in the autonomic nervous system innervating the heart, contributing to both neuronal excitability deficits in cardiac anomalies related to ASD.

Dissecting the impact of endolysosomal Ca^{2+} dynamics on synaptic function and plasticity

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Presynaptic terminals in the hippocampus are essential for synaptic transmission, and maintaining intracellular calcium (Ca^{2+}) homeostasis is critical for their proper function. Recent studies have demonstrated that Ca^{2+} stores can dynamically adjust between release and buffering roles in response to environmental changes. However, the involvement of highly motile endo-lysosomal Ca^{2+} stores in presynaptic performance remains underexplored.
In this study, we investigate endolysosomes containing the Ca^{2+}-release channels, Transient Receptor Potential Mucolipin 1 (TRPML1) and Two-pore Channel 2 (TPC2), within the mouse hippocampus. Using immunofluorescence staining, we identify a stable subpopulation of endolysosomes localized to presynaptic compartments, which increases under various paradigms of neuronal activation. These findings suggest that endolysosomes play a localization-dependent and physiologically significant role at presynaptic boutons, highlighting their dynamic contribution to meeting the homeostatic demands of synaptic activity.
By employing live Ca^{2+} imaging, we further demonstrate that neuronal activity induces Ca^{2+} release from endolysosomal stores, contributing to the maintenance of presynaptic Ca^{2+} homeostasis. This suggests that central synapses may utilize multiple Ca2+-dependent mechanisms for presynaptic control of ongoing synaptic activity, thus arbitrating properties such as the neurotransmitter release probability. Additionally, pharmacological manipulation reveals that endolysosomal Ca^{2+} stores regulate hippocampal presynaptic maintenance through distinct Ca^{2+}-release strategies highlighting that endolysosomal Ca^{2+} signaling might influence neuronal excitability and long-term processes, such as synaptic plasticity.
Taken together, our results provide new insights into the role of endolysosomal Ca^{2+} in presynaptic function. These findings may help elucidate the contribution of Ca^{2+} dysregulation in the pathophysiology of various neurological disorders, where disruptions in endolysosomal Ca^{2+} signaling are implicated.

Early-life inflammation alters microglia phenotypes and attenuates amyloid-beta pathology

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Early infection in life has been implicated in increasing the risk for neurological disorders. Our study aims to investigate how systemic inflammation affects the immune system of the central nervous system (CNS) and what early alterations occur in microglia and monocytes that may lead to destructive effects in CNS. We also attempt to elucidate the underlying mechanisms and unique profiles of these myeloid cells in wild type (WT) and an AD mouse model (5xFAD). We performed single-cell RNA sequencing of microglia in the brain and monocytes in the bone marrow from 6-month-old WT and 5xFAD mice subjected to LPS injection at postnatal period. We successfully mapped disease-associated microglia (DAM) and perivascular macrophages in our data. Additionally, we observed LPS induced a distinct population of microglia in WT mice and primed microglia into a standby-DAM state upon Aβ pathology. In the periphery, we found MMP-9 was significantly increased in serum samples from 5xFAD mice. Interestingly, early LPS stimulation significantly elevated TNF-α in serum from WT and 5xFAD mice. Novel objective recognition tests showed that LPS treatment in 5xFAD mice had a tendency to improve the short-term memory deficit. Taken together, we observed long-lasting effects from early-life stress/immune response, including activation of inflammation in both the periphery and brain through modulation of different signaling cascades.

Early-life stress impairs microglial adaptability in the periaqueductal grey of Mecp2-heterozygous mice

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Mutations in MECP2 lead to Rett syndrome (RTT), a severe neurodevelopmental disorder characterised by altered stress responses, heightened anxiety, and impaired pain processing. Increasing evidence suggests that early-life stress (ELS) exacerbates the neurodevelopmental deficits in Mecp2-deficient mice, yet its impact on microglial plasticity in the associated brain regions remains poorly understood. The periaqueductal grey (PAG) is a key structure for pain modulation, autonomic control, and defensive behaviours, all of which are disrupted in RTT. Here, we examined whether ELS affects microglial morphology in the PAG of presymptomatic Mecp2-heterozygous (Mecp2-het) female mice. Using maternal separation (MS) as an ELS model, we assessed IBA1-immunoreactive microglia in the dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG), and ventrolateral PAG (vlPAG) through morphometric and fractal analyses.

While global PAG analysis showed no significant differences in IBA1 expression levels, subdivision-specific analyses revealed microglial alterations. In the dmPAG, Mecp2-het mice exposed to MS exhibited increased branching and endpoints per microglial cell when compared to wild-types (WT, $p < 0.05$). Thes changes indicate that, while microglia attempted to extend their processes in response to MS, their overall morphology remained more compact and less complex compared to WT. This suggests an ineffective or maladaptive response to environmental adversity. In the dlPAG, MS significantly reduced microglial branch length in WT mice ($p < 0.05$), but not in Mecp2-het mice, indicating impaired stress-induced plasticity. In the vlPAG, fractal analysis revealed reduced circularity in Mecp2-het-MS microglia ($p < 0.05$), suggesting a maladaptive ramified state.

These results indicate that Mecp2-deficiency compromises microglial responsiveness to ELS in a region-specific manner. The failure of microglia to appropriately remodel their morphology may contribute to the altered stress coping strategies, anxiety alterations, and sensory deficits observed in RTT, reinforcing the importance of microglial adaptability in neurodevelopmental disorders.

Effect of Nanoplastics on Pro-inflammatory Activation Induced by Airborne Particles in Microglial Cells

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Micro- and nanoplastics (NPs) have emerged as a class of environmental pollutants over the last decade. These particles have been detected globally across diverse ecosystems and in various organisms, including humans. Recent studies have reported the accumulation of microplastics, particularly in the human brain, raising a big concern regarding their potential adverse effects. The present study aims to investigate the impact of NPs on the pro-inflammatory activation of microglial cells and their potential interaction with airborne particulate matter (PM), another critical environmental contaminant.

In order to achieve this objective, an in vitro study was conducted using the BV2 microglial cell line. Cell viability following exposure to NPs and PM at different concentrations, as well as their co-exposure, was assessed using the MTT assay. Furthermore, gene expression levels of key pro-inflammatory markers and neurotrophic factors were quantified via RT-qPCR. The activation of the NF-κB transcription factor and the expression of proteins involved in autophagy and oxidative stress pathways were also evaluated through Western blot analysis.

The results demonstrated that NPs did not significantly upregulate pro-inflammatory marker expression at low concentrations. In contrast, PM exposure at low doses significantly increased the expression of pro-inflammatory markers. Notably, co-exposure to both pollutants at low doses resulted in a reduction in pro-inflammatory marker expression compared to exposure alone. This modulatory effect of NPs on PM-induced pro-inflammatory responses was linked to the ability of NPs to influence autophagic processes in microglial cells.

These findings suggest that NPs may modulate the effects of PM on microglial cells by attenuating their pro-inflammatory response and influencing the expression of neurotrophic factors. This modulation indicates a potential interference of NPs with the neuroprotective functions of microglia in response to PM exposure. However, further studies are required to elucidate the mechanistic basis of NP-mediated modulation of PM-induced toxicity in microglial cells.

Electrophysiological alterations induced by Lis1 mutation in somatostatin-expressing GABAergic interneurons of the cingulate cortex

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The Lis1 gene regulates dynein-dependent axonal transport, mediates neuronal migration during brain development, and maintains synaptic integrity in the mature brain. Lis1 is expressed throughout the lifespan of the central nervous system, and in humans, defects in LIS1 expression lead to type I lissencephaly.
We have investigated the electrophysiological alterations resulting from selective Lis1 inactivation in somatostatin-expressing cortical GABAergic interneurons (SST^+ neurons). To achieve this, we generated a conditional Lis1 knockout-RFP mouse (Lis1 cKO-RFP under somatostatin-Cre driver: mutant animals); as controls we used mice expressing RFP under somatostatin-Cre driver (wild-type animals). The neuronal electrophysiological properties were examined in layer 2/3 of the cingulate cortex, specifically in the anterior cingulate cortex (ACC) and retrosplenial cortex (RSC), using coronal brain slices from P14–P21 mice. Whole-cell patch-clamp recordings were performed at 32–33°C with standard intracellular and extracellular solutions.
We identified statistically significant electrophysiological differences between wild-type and mutant SST^+ neurons (n=14-38 neurons; Mann–Whitney test for comparisons). Some differences were consistent across ACC and RSC neurons: Lis1 mutant SST^+ neurons exhibited a more depolarized resting membrane potential, greater time-dependent rectification during hyperpolarizing responses, and prolonged action potential duration. In contrast, we observed region-specific alterations: Lis1 mutant SST^+ neurons displayed an increased firing frequency in response to depolarizing current pulses only in ACC, whereas these neurons exhibited a higher input resistance only in RSC.
These results demonstrate that Lis1 is essential for the proper electrophysiological function of SST^+ neurons, and suggest that Lis1 inactivation leads to dysfunction in cortical circuits due to the resulting electrophysiological impairments in this population of inhibitory neurons. Also, the data suggest the presence of differences among cortical regions with respect to the consequences of Lis1 gene dysfunction.
Funding: MINECO/AEI/FEDER (PID2020-11817RB-100 and PID2023-150680OB-I00), GVA (PROMETEO-CIPROM/2021/018). The Institute of Neurosciences is a “Centre of Excellence Severo Ochoa” (CEX2021-001165-S).

Epileptiform discharges trigger morphological and electrophysiological changes on hippocampal newborn neurons

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Epileptiform activity (EA) consists of abnormal excessive neuronal discharges (spikes and sharp waves) that can be detected in the electroencephalogram. Although originally associated with epilepsy, EA is also present in many other conditions such as Alzheimer's disease, Parkinson's disease, Autism Spectrum Disorder, dementia, traumatic brain injury and aging. Indeed, increasing evidence suggests that EA may contribute to the cognitive impairments observed in these conditions, including deficits in memory, learning, decision-making, and mood regulation.

The precise neurobiological mechanisms linking EA to these behavioral deficits remain unclear; however, adult hippocampal neurogenesis has emerged as a potential underlying mechanism. Using a pure model in which EA is induced by a single intrahippocampal injection of kainic acid, we investigated structural and functional changes in hippocampal immature neurons born at the time of EA induction or born two months afterward. EA induced morphological alterations in newborn neurons at both time points, such as abnormal migration and more complex dendritic arborization with increased dendritic spine density.

To determine whether these morphological changes translate into functional alterations we performed whole-cell patch-clamp recordings on retroviral vector-labeled newborn neurons. Our data reveal that EA newborn neurons exhibit altered electrophysiological properties also at the two time points of analysis. Specifically, EA newborn neurons show an increased membrane potential and capacitance. Additionally, EA newborn neurons display a greater action potential rheobase and altered firing rate in response to current injections, and increased afterhyperpolarization.

Altogether, we conclude that EA induces morphological and electrophysiological changes in hippocampal newborn neurons at short and long term. In order to investigate whether a standard-of-care treatment for hyperexcitation (levetiracetam) preserves the structure and function of newborn neurons in our EA model, we are currently treating EA mice with levetiracetam for 15 days using osmotic minipumps.

Excitatory-inhibitory imbalance at Purkinje cells after N-glycosylation impairment

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Alterations in N-glycosylation pathway lead to Congenital Disorders of Glycosylation (CDG), a group of diseases that include cerebellar dysfunction causing severe ataxia. Despite the high prevalence of neurological disorders associated with CDG, N-glycosylation modulation of nervous system proteins has been largely unexplored. Traditionally, N-glycosylation -a universal protein post-translational modification mechanism- has been associated with protein folding and surface expression. However, recent studies have demonstrated that N-glycosylation also modulates protein function, including proteins localized at cerebellar synapses.

We hypothesized that the disruption of N-glycosylation affects Purkinje cell (PC) synaptic inputs and cerebellar function. To investigate the synaptic role of N-glycosylation, we used cerebellar organotypic cultures and applied the inhibitor Kifunensine (Kf) to block N-glycosylation maturation. After impairing glycosylation, alterations in Purkinje cell (PC) firing rate and spontaneous inputs were observed, altogether disrupting the cerebellar excitatory/inhibitory (E/I) balance by favouring the inhibitory transmission. Importantly, these findings are consistent with previous reports from ataxia mouse models.

Complementary to the electrophysiological study we performed an immunolabeling of inhibitory synapses with VGAT and Parvalbumin in cultured cerebellar slices. The study revealed an altered pattern of inhibitory synapses and nuclei in the cerebellar cortex after glycosylation impairment, with a reduction of VGAT puncta in the upper molecular layer and decreased density of parvalbumin-positive cells.

Based on this evidence, we propose that N-glycosylation plays a distinct role in modulating the function of excitatory and inhibitory synapses in the cerebellar circuit. Disruption of this process could lead to imbalances in synaptic transmission leading to altered PC inputs, aberrant PC firing patterns and ultimately ataxia and other cerebellar symptoms observed in CDG patients.

Extracellular vesicles from peripheral blood mononuclear cells of hyperammonemic rats induce neuroinflammation in hippocampus, impairing memory and learning when injected into normal rats

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Patients with liver cirrhosis may show minimal hepatic encephalopathy (MHE), with mild cognitive impairment. Studies in patients and animal models show that MHE is triggered by a shift in peripheral inflammation. Peripheral extracellular vesicles (EVs) seem to mediate transmission of deleterious effects to brain. However, it is not known which cell types produce the pathological EVs. The aim of this study was to assess if EVs released from cultured peripheral blood mononuclear cells from hyperammonemic rats with MHE (HA-PBMC-EVs) transmit pathological signals to the brain when injected to normal rats and to analyze the underlying mechanisms.

HA-PBMC-EVs were isolated from primary PBMCs cultures of hyperammonemic rat and were injected into control rats to study cognitive function and neuroinflammation in hippocampus. We used ex vivo experiments to investigate the mechanisms by which HA-PBMC-EVs from hyperammonemic rats induce neuroinflammation and alter neurotransmission in hippocampal slices from control rats.

We found that HA-PBMC-EVs induce neuroinflammation in hippocampus and impair memory and learning in control rats. HA-PBMC-EVs enhance membrane expression and activation of TNFR1 and of S1PR2, increasing IL-1β and phosphorylation of Src which increases CCL2 and BDNF content and membrane expression of the NR2B subunit of the NMDA receptor. BDNF activates TrkB leading to phosphorylation and activation of Akt, which further increases BDNF. The increased activation of NR2B alters membrane expression of GluA1 and GluA2 subunits of AMPA receptors, leading to alteration of neurotransmission which would be responsible for the induction of cognitive impairment. All these effects of HA-EVs were prevented by blocking TNFα, with infliximab, indicating that they were mediated by enhanced activation of TNFR1 by TNFα. EVs from PBMCs may be therefore a therapeutic target on which to act to reverse cognitive impairment in patients with MHE.

Extrasynaptic tonic GABAergic inhibition scales with the volume of the brain extracellular space

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Volume transmission between neural cells is commonly thought of as homogenous and slow in nature, with differences in signal strength between neurons attributable to differences in receptor numbers or states, or to general differences in neuronal excitability. Volume transmitted signals derived from point-source release, which is then balanced by uptake mechanisms so that (all other things equal) there is a steady state number of molecules in the extracellular space. Accordingly, volume transmission is commonly investigated uniquely from the stance of these parameters: receptors and uptake mechanisms. However, this scenario ignores that the transmitter concentration around a given point source will scale with the local extracellular space volume, and this simple volume-dilution effect will putatively be valid across nano- to mesoscales. We hypothesize that extrasynaptic tonic GABAergic inhibition will scale with the volume of the extracellular space.
To investigate this, we have applied whole-cell patch-clamp recordings to measure tonic GABAergic inhibition from hippocampal CA1 neurons in mouse brain slices under normal and hyperosmotic conditions that effectively shrink cells and expand the extracellular space. Volume changes were confirmed by 2-photon microscopy in live slices. Hyperosmotic conditions induced an extracellular volume expansion of around 20% with no change in synaptic GABA signalling, while tonic inhibition decreased around 40%.
We demonstrate that extrasynaptic tonic GABAergic inhibition scales with the extracellular space volume, and that extracellular space volume should be considered an additional regulator of signalling that is independent of both the transmitting and receiving neural cells and cannot be investigated by focusing on these alone. This cell-independent signaling regulation mechanism will be interesting to investigate further as it could be a regulator of networks that act on slower time scales that synaptic signals and beyond synaptically defined networks.

Frequency-Dependent Effects of tACS on Pyramidal Cells and Interneuron Synchronization

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Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation technique that modulates neuronal activity through subthreshold electric fields, leading to frequency- and intensity-dependent neuronal entrainment. While initially thought to primarily affect pyramidal cells due to their morphology, recent findings highlight the importance of network interactions, with interneurons also contributing to frequency-specific modulation. This study examines the effects of 10Hz and 40Hz-tACS (±10 to ±50µA, in 10µA steps) on neuronal activity in the primary somatosensory cortex of awake mice.

Extracellular recordings were obtained from 11 C57BL/6 mice, capturing activity from 302 neurons (216 pyramidal cells, 86 interneurons) using high-density electrophysiology system Neuropixels 1.0. Spike sorting and classification were performed with Kilosort and CellExplorer. tACS effects were assessed via Phase-Locking Value (PLV), comparing baseline (5s pre-tACS) and stimulation (5s tACS). PLV modulation varied across neuronal types and stimulation conditions. In interneurons, 10Hz-tACS induced a decrease in PLV at lower intensities (maximum decrease at 10µA, 78.57%±6.42%, SEM), whereas 40Hz-tACS led to an increase (maximum effect at 50µA, 198.89%±19.13%, SEM). In contrast, pyramidal cells exhibited PLV enhancement under both frequencies (maximum increase at 50µA, 125.01%±5.76%, SEM for 10Hz, 130.01%±6.81% for 40Hz). When comparing frequencies, interneurons displayed stronger synchronization at 40Hz-tACS, whereas pyramidal cells showed no frequency-dependent differences. Comparing neuronal populations, pyramidal cells showed stronger phase-locking at 10Hz, while at 40Hz-tACS, interneurons exhibited the highest PLV value.

These findings indicate that tACS effects vary according to both frequency and intensity, with interneurons displaying greater sensitivity to 40Hz-tACS, while pyramidal cells require higher intensities to engage in phase-locking at either frequency. While 10Hz- and 40Hz-tACS elicited comparable entrainment in pyramidal cells at high intensities, interneurons exhibited fundamentally different responses to these frequencies. These entrainment patterns highlight the role of network dynamics in tACS-induced synchronization. Understanding these effects is key to optimizing neuromodulation strategies.

Functional astro-neuronal networks associated to direct and indirect pathway are disrupted in a Parkinsonism model

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Astrocytes show a form of excitability based on intracellular calcium variations. They can be activated by synaptically released neurotransmitters and they in turn release gliotransmitters that regulate synaptic transmission. Combining calcium Ca^{2}+-imaging techniques and whole-cell recordings of molecularly identified medium spiny neurons (MSNs) from dorsal striatum, we have demonstrated the existence of functional astro-neuronal networks in the striatum that comprise subpopulations of astrocytes, neurons and synapses belonging either to the direct or the indirect basal ganglia pathways (Martín et al., Science 2015). We have investigated the synaptic consequences of the potential dysregulation of these astro-neuronal networks in an animal model of Parkinson's disease by treating mice with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP-treatment reduced the astrocyte Ca^{2}+ responsiveness to endocannabinoids released by MSN stimulation and abolished the astrocyte-mediated heteroneuronal potentiation between homotypic MSN. MPTP treatment did not compromise astrocytic Ca^{2}+ mobilization machinery or the ability of MSNs to release endocannabinoids, because pharmacologically-evoked astrocytic Ca^{2}+ responses and the homoneuronal depolarization suppression of excitation remained unaffected. Furthermore, direct stimulation of astrocytes with ATP was able to induce the mGluR5/1-mediated synaptic potentiation in MPTP-treated mice. These results indicate that some signaling properties in astro-neuronal networks are disrupted in an animal model of Parkinson's disease, suggesting that disturbances in the astrocyte-neuron communication may be involved in neurodegenerative diseases.

FUNCTIONAL GENETIC ANAYLIS OF SGT-B IN SYNAPTIC MAINTENANCE IN THE MOUSE BRAIN

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Cysteine String Protein alpha (CSPα/DNAJC5) is a key co-chaperone, located in the synaptic vesicles and supports neurotransmitter release by stabilizing the SNARE complex. CSPα is known to interact with Hsc70 and Small Glutamine-Rich Tetratricopeptide Repeat-Containing (SGT) proteins, but the precise function of SGT proteins in synaptic maintenance remains unclear. We hypothesize that if CSPα requires direct interaction with SGT proteins for its function, eliminating SGT proteins might replicate the phenotype observed in CSPα knockout (KO) mice. To investigate this, we generated an SGTB knockout mouse model. Unexpectedly, these mice did not exhibit neurodegeneration and maintained normal weight, motor function, and lifespan. However, inhibitory neurons showed subtle region-specific changes: CSPα particle density decreased in the dentate gyrus. Moreover, the cerebellum was also analyzed, particularly axo-somatic synapses between Purkinje cells (PCs) and Large Glutamatergic Neurons (LGNs), but no significant differences were observed in SGTB KO mice. Interestingly, previous studies showed that SGTA deletion alone did not cause neurodegeneration, suggesting that SGTA and SGTB share redundant functions. To explore this, we generated a double knockout (DKO) mouse model lacking both SGTA and SGTB isoforms. The viability of these mice was dramatically compromised and the mice that were born presented perinatal lethality, supporting the notion that both proteins have redundant but vital functions. Further experiments are required to demonstrate if the vital function of these proteins is linked or not the function CSPα/DNAJC5 at the nerve terminals.
Funding: Spanish Agencia Estatal de Investigación and Ministerio de Ciencia, Innovación y Universidades and European Regional Development Fund (ERDF), (MICIU/AEI /10.13039/501100011033,FEDER, UE) project PID2022-138957NBI00 and PRE2020/095166, University of Seville, (Plan Propio de Investigación; Spanish Science, Technology, and Innovation System; Contrato Acción II.4 PPIT -Plan de Captación y Retención de Talento-), the Andalusian Consejería de Salud y Consumo (RH-0064-2021) and the Spanish Instituto de Salud Carlos III (ISCIII).

Genetic dissection of GluN3A-mediated stress resilience

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Chronic stress affects brain structure and function and is a major risk factor for neuropsychiatric disorders including post-traumatic stress disorder, anxiety and major depressive disorder (MDD). A principal neural adaptation is a long-lasting alteration in synaptic connectivity that is thought to be caused by impaired local protein translation. The mammalian target of rapamycin complex 1 (mTORC1) is a key determinant of translation, and current efforts are centered in therapeutics that foster mTORC1 activation—a prime example is the NMDAR antagonist ketamine. Recent work implicates the GluN3A non-conventional NMDA receptor subunit,as a key regulator of mTORC1 signaling at synaptic loci. Yet to date efforts have focused on targeting classical NMDA receptors, and GluN3-NMDARs have been overlooked despite selective expression in brain regions critical for stress regulation such as the prefrontal cortex, ventral hippocampus or amygdala. Using chronic stress models, we now report that deletion of GluN3A in male and female mice confers stress resistance tested in a variety of paradigms (light-dark box, elevated plus maze and tail suspension test). Stress resistance of GluN3A KO mice was associated with stronger mTORC1 activation levels in basal conditions and upon chronic stress, as shown by enhanced or reduced phosphorylation of the mTORC1 downstream targets S6 and eEF2. Conversely, overexpression of GluN3a increased anxiety-like and depressive-like behaviors. Using conditional knockout GluN3A mice, we identified excitatory neurons in prefrontal cortex as the locus for GluN3A modulation of stress responses. At the mechanistic level, we found that GluN3A and mTORC1 machinery are altered in MDD patients and chronic stressed mice models, suggesting that GluN3a might be a new promising therapeutical target for chronic stress-related disorders.

GluN2A-dependent ionic currents and calcium signaling in human iPSC-derived neurons

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The maturation of excitatory neurons in the human cerebral cortex depends on proper glutamatergic neurotransmission, primarily mediated by N-methyl-D-aspartate receptors (NMDARs). A critical developmental shift occurs from GluN2B to GluN2A subunits, influencing neuronal function and synaptic maturation. Disruptions in this transition have been implicated in neurodevelopmental and neurodegenerative disorders.
Here, we characterize neural cultures differentiated from iPSCs under two different conditions: BrainPhys Neuronal Medium (BPM) and Neural Maintenance Medium (NMM), employing transcriptomics, confocal microscopy, patch-clamp electrophysiology, and calcium imaging to examine the functional consequences of the GluN2B-to-GluN2A switch in vitro.
We show that BPM cultures exhibited a 1:1 neuron-to-astrocyte ratio, thus promoting a neuron-to-astrocyte ratio closer to human brain physiology, compared to 8:1 in NMM cultures. RNA sequencing of neural cultures identified 1,924 differentially expressed genes when comparing both conditions and the gene set enrichment analysis revealed upregulation of pathways associated with neuronal differentiation and synapse formation in BPM cultures, while astrocyte-associated genes were also significantly increased. Maturation of iPSC-derived neurons was further assessed by analyzing NMDAR localization and function. Immunostaining demonstrated a nine-fold increase in synaptic NMDARs from day 30 to day 60 in culture conditions, with a higher expression in BPM cultures. Gene expression analysis revealed a shift from GRIN2B to GRIN2A subunits, indicating neuronal maturation. Whole-cell patch-clamp recordings confirmed functional NMDAR-mediated ionic currents, with GluN2A being the predominant subunit in BPM-derived neurons. Calcium imaging showed that NMDA stimulation elicited robust intracellular calcium increases, significantly greater in BPM cultures, suggesting enhanced calcium regulation in BPM cultures.
In conclusion, we have demonstrated that iPSC-derived neurons exhibit progressive NMDAR synaptic incorporation and a GluN2B-to-GluN2A switch, aligning with developmental maturation. Our findings highlight differences between differentiation protocols, with BPM cultures achieving greater synaptic maturity. These insights underscore the importance of modeling neuronal development accurately, particularly for aging-related disorders and neurodegeneration research.

Glutamate regulates lipid droplets accumulation and fatty acid metabolism in astrocytes

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Energy metabolism in the brain accounts for approximately 20% of the body's oxygen consumption, with glutamatergic transmission being a major contributor. To meet these energetic requirements there is a tight coupling between neurons and astrocytes. Thus, neuronal glutamatergic activity decreases astrocytic oxidative glucose metabolism in favor of glucose metabolism to lactate. However, little is known about the interplay between neuronal activity and astrocytic metabolism concerning other energetic sources. Here, we used cultured astrocytes, fluorescence microscopy and a wide range of biochemical assays to show that glutamatergic neurotransmission also decreases mitochondrial fatty acid beta-oxidation and oxygen consumption, thereby increasing the local oxygen availability. Mechanistically, glutamate inhibition of fatty acid metabolism involves GLAST/EAAT1 transporters but not activation of glutamate metabotropic nor NMDA or AMPA receptors. Moreover, glutamate prompts a reduced metabolic state without significant changes in ATP levels. Importantly, this glutamatergic-induced metabolic adaptation enhances lipid droplet accumulation and ensures cell survival. In conclusion, glutamate regulation of astrocytic use of glucose and fatty acids provides the brain with metabolic flexibility able to perfectly couple and fulfill astrocytic functions.

Impact of environmental enrichment on adult CA3 cytoarchitecture: new mossy fibers and microglia

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The hippocampal CA3 region undergoes continuous synaptic remodeling and incorporates new axons, known as mossy fibers, as a consequence of adult neurogenesis. Environmental enrichment (EE) strongly influences hippocampal function by promoting neurogenesis and synaptogenesis. In this study, we investigate the effect of EE on the CA3 region, focusing on new mossy fibers and microglia, cells involved in axogenesis and connectivity modulation.
Using stereological techniques, we found that EE increased CA3 volume. Since EE enhances adult neurogenesis, we hypothesized that a proportional increase in axons reaching CA3 should contribute to its enlargement. However, when we analyzed the new mossy fibers using PSA-NCAM labeling, we did not detect any changes in the space they occupied in EE mice. These findings suggest that CA3 enlargement and EE-induced adult neurogenesis are not directly related. To explore alternative mechanisms, we are analyzing the effects of EE on vGlut1-positive glutamatergic terminals.
Additionally, our microglia analysis showed that EE reduced the space they occupy without altering their number, suggesting a decrease in their surveillance activity within CA3. Thus, we are now analyzing if EE alters microglial surveillance of new axons and/or presynaptic terminals.
Our study will provide new insights into the cellular mechanisms underlying EE's impact on hippocampal function.
Funding: Science and Innovation Spanish Ministry (PID2022-140525NB-I00 and PID2022-140456NB-I00). Erasmus+ programme Partnership for Cooperation (2023-1-PL01-KA220-HED-000160284). Strategic plans and strategic research programmes of excellence from the Regional Government of Castile and León, co-funded by the ERDF Operational Programme (CLU-2023-1-01).

Impact of Maternal Separation and Post-Weaning Isolation on Behavioral and Physiological Outcomes in a Murine Double-Hit Model

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Exposure to aversive experiences during early life has been associated with alterations in brain maturation and may play a role in the development of psychiatric disorders such as major depression or schizophrenia. Previous animal models have observed changes in behavior linked to these early-life stressors, which coincide with key neurodevelopmental processes occurring during the early postnatal period in mice, such as the closure of critical periods of inhibitory circuits. The alterations induced by early stress in these circuits have been well described in the cortex, but are not well understood yet in other subcortical regions, such as the thalamus. This diencephalic region is very relevant for the processing of sensory information and different higher cognitive functions, and its physiology and structure are altered in some psychiatric disorders. Interestingly, the flux of information in the thalamus is tightly controlled by a population of inhibitory neurons concentrated in the thalamic reticular nucleus (TRN). This study aims to explore, using a murine double-hit model, how early-life adversities influence behaviors dependent on the thalamus and specifically the TRN and different structural and physiological parameters related to the thalamic inhibitory circuitry. The model combines as an early-life stressor maternal separation (MS) from Postnatal day 7 (P7) until weaning (P21) with post-weaning social isolation as a chronic stressor. Adult male and female mice were evaluated for various behavioral tests, including open field and sucrose preference, or weight gain over time. Preliminary results show reduced weight gain during the MS period, especially in females. Adult animals did not show a significant difference in sucrose preference compared to water nor weight gain but displayed an increase in locomotor and anxiety-related behaviors, especially in females. We are also conducting molecular and cellular analyses focused on thalamic inhibitory neurons and on molecules known to regulate their physiology and plasticity.

Impact of mechanical stimulation on K2P channels in the Intracardiac Ganglia of mouse, an in vitro study

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The intrinsic cardiac nervous system, a complex circuit composed by the intracardiac ganglia (ICG), serves as the heart's final integration center, regulating local cardiac performance. ICG neurons express functional TREK channels, two-pore potassium channels responsible for the maintenance of neuronal resting membrane potential and excitability. These channels respond to various physiological stimuli, including mechanical stress, indicating their potential role in mechanoelectrical feedback in the heart. Here we investigated how ICG neurons respond to mechanical stimulation and discovered a decrease in electrical activity, partially attributed to the activation of TREK-like channels, suggesting that mechanical stimulation influences these channels ultimately impacting neuronal activity. Our findings indicate a possible neuroprotective role of TREK channels in the ICG during conditions such as ischemia.

Influence of environmental enrichment on microglial surveillance of dendrites of immature hippocampal neurons

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Physical, social and cognitive stimulation provided by an active lifestyle induces changes in the structure and function of the brain that promote healthy aging. This phenomenon has been widely studied in the hippocampus, as it is a brain structure that exhibits high plasticity, including the production of new neurons in the dentate gyrus during adulthood (adult hippocampal neurogenesis: AHN).
Previous studies have focused mainly on the effects of environmental stimulation on the production of new neurons. However, the cellular mechanisms by which lifestyle modulates AHN are not entirely understood yet. In this study, we investigated the effect of environmental stimulation on microglía within the molecular layer of the dentate gyrus, as microglia have the potential to detect changes in its microenvironment and consequently modulate AHN. We housed two-month-old mice in an enriched environment (environmental enrichment: EE) for six weeks and we compared them with animals housed in standard conditions (non-enriched: NE).
Our results indicate that EE increases the size of the dentate gyrus and the dendritic density of immature neurons located in the molecular layer. Additionally, EE reduces the area occupied by microglia in the molecular layer without changing their number. Furthermore, our colocalization analyses suggest that EE decreases microglial surveillance of new dendrites. These findings indicate that EE affects the interaction between microglia and newly generated neurons.

Funding: Science and Innovation Spanish Ministry (PID2022-140525NB-I00 and PID2022-140456NB-I00). Erasmus+ programme Partnership for Cooperation (2023-1-PL01-KA220-HED-000160284). Strategic plans and strategic research programmes of excellence from the Regional Government of Castile and León, co-funded by the ERDF Operational Programme (CLU-2023-1-01). Aids for University Teacher Training from the Science, Innovation and Universities Spanish Ministry (MICINN; FPU23/00987).

Investigating the role of astrocytes in sharp-wave ripple function

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Sharp-wave ripples (SPW-Rs) are hypersynchronous activity patterns in the mammalian hippocampus known to replay neuronal sequences related to past experiences and are crucial to memory function. SPW-Rs emerge from temporally and spatially coordinated activity, involving a significant release of neurotransmitters and neuromodulators, exquisitely orchestrated across nearly every neuron type in the hippocampal circuit. Astrocytes, active elements of brain circuits, express receptors of neurotransmitters and neuromodulators that sense neuronal activity. The activation of these receptors leads to an increase in intracellular calcium levels that in turn trigger the release of active molecules (gliotransmitters) that impact neuronal activity. This bidirectional communication between astrocytes and neurons gave rise to the concept of the tripartite synapse and has been demonstrated to affect memory processes and animal behavior. However, the role of astrocyte activity in SPW-R function, a neuronal signature of memory consolidation, has not yet been determined. Therefore, to investigate the relationship between neurons and astrocytes during SPW-Rs we simultaneously monitored hippocampal neuronal and astrocyte activity in freely moving mice. We recorded neuronal activity using silicon probes and the population astrocyte calcium activity using fiber photometry. Recordings were acquired during a hippocampal-dependent spatial memory paradigm (alternation task) and sleep periods before and after the task. This allowed us to evaluate network changes as mice learned the paradigm across training days. Furthermore, we applied an in-lab classifier to discern between different hippocampal neuronal subpopulations. Using this experimental design, we investigated the dynamics and interplay between astrocyte calcium levels and the activity of specific neuronal subpopulations in SPW-Rs. This approach revealed distinct relationships between astrocytes and specific neuronal populations during SPW-Rs across learning. These results suggest the involvement of a non-neuronal population in SPW-R function and expand our understanding of the role of astrocytes in circuit activity regulation and memory.

LPS and IFNγ elicit distinct cellular functions in a tri-culture of murine astrocytes and human iPSC-derived dopaminergic neurons and microglia

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Parkinson's disease (PD) is a chronic, progressive, and complex neurodegenerative disorder characterized by the accumulation of α-synuclein (α-Syn) and glial dysregulation. To better understand the interplay between them, we generated induced pluripotent stem cells (iPSCs) derived dopaminergic neurons and microglia, and co-cultured them with murine astrocytes producing an advanced tri-culture model for PD. To investigate the interaction between aggregation, microglial response, and neuronal health/survival this tri-culture was treated with: α-Syn pre-formed fibrils (PFFs), together with Lipopolysaccharide (LPS) or interferon γ (IFNγ).
We found that PFF and LPS reduced the stained area by tyrosine hydroxylase (TH) while IFNγ treatment provided a significant rescue of TH levels back to control levels. This was also reflected in an increase in the synaptic apposition area in IFNγ treated cells, indicating an improvement in the established connections between neurons. Microglia showed an expected increase in the number of cells after IFNγ treatment concomitant with a restoration of control lysosomal counts per cell. To understand whether the observed effects on the tri-culture were due to the response of microglia to treatments, we decided to investigate a monoculture of microglia treated acutely and chronically with PFFs and LPS/ IFNγ.
When treated with PFFs, acute and chronic IFNγ treatment led to a decrease in lysosomal area and the α-syn area within lysosomes, indicating increased α-Syn degradation. However, acute and chronic LPS treatment produced a dramatic decrease in phagocytic capacity in both vehicle and PFF treated cells, which was accompanied by an increase in the lysosomal area of the cells comparable to that of PFF-treated microglia, and an increase in the α-Syn area within lysosomes, indicating decreased a-Syn degradation.

Overall, all these results indicate that LPS and IFNγ affect microglial function distinctly, and that glial cell communication plays a crucial role in neuronal survival.

Macromolecular GIT1/βPIX condensates define mTOR localization and signaling at synapses

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Control of local protein synthesis at subsets of synapses is essential for experience-dependent plasticity and memory formation. A key regulator of translation efficiency is the mechanistic target of rapamycin complex 1 (mTORC1), one of the two known mTOR complexes in mammalian cells, composed by the mTOR kinase and its regulator Raptor. mTORC1 exerts its function in multiple cellular compartments in response to a variety of cell signals. Previous work has unravelled the identity of molecular adaptors that nucleate mTOR signaling in lysosomal compartments, but how mTORC1 is nucleated at synaptic loci is unknown. Here we identify GIT1 and βPIX macromolecular complexes as critical organizers of mTORC1 recruitment and activation to synapses, and map the underlying molecular mechanisms. We begin by demonstrating that GIT1 facilitates mTOR localization to dendritic spines via its synaptic localization domain (SLD). However, the SLD domain alone is not sufficient for mTOR recruitment suggesting a role of additional cellular partners. We then show that the interaction between GIT1 and its partner βPIX, as well as the formation of GIT1/βPIX macromolecular condensates, are necessary for proper mTOR synaptic targeting. Disrupting condensate formation through mutations that prevent multimerization of GIT1 or βPIX impairs mTOR localization and mTORC1 activation by synaptic signals. Specifically, we find that BDNF- and chemical LTP-induced mTOR activation is dependent on the integrity of the GIT1/βPIX complex.

Our findings highlight GIT1/βPIX condensates as essential cellular platforms for the spatial regulation of mTORC1 signaling at synapses. More broadly, this work supports the role of biomolecular condensates in organizing key components of synaptic signaling pathways, ensuring precise control of local protein synthesis during synaptic plasticity.

Manipulation of astrocytes with neurotoxic potential in spinal cord as a therapeutic target for motor and functional recovery after incomplete spinal cord contusion injury

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Spinal cord injury (SCI) is a pathology that still lacks effective treatments to improve the quality of life of patients. Previous studies have shown that after SCI, microglia adopt a protective phenotype by eliminating dead cells, as well as participating in synaptic remodelling. However, microglia, through the joint release of IL-1α, TNFα and C1q, are also the trigger for the polarization of astrocytes towards a neurotoxic phenotype capable of decreasing axonal regeneration and decreasing synaptic connectivity.

Based on this, the present study hypothesized that inhibition of neurotoxic astrocytes (positive for complement component C3) could favour the recovery of neuronal connectivity after SCI by modulating the death of axotomized neurons. Therefore, the main objectives of this work are focused on 1) initial characterization of the presence of C3+ astrocytes in the spinal cord after moderate contusion, 2) determining their anatomical relationship with the ascending sensory and descending motor tracts, 3) determining how pharmacologic inhibition of C3+ astrocytes contribute to functional recovery of locomotion, balance, and motor coordination in SCI mice.

Our results indicate that moderate SCI increases the number of C3+ astrocytes near corticospinal tract axons in the rostral zone, whereas in the caudal zone, they are primarily localized to the reticulospinal tracts. These findings suggest a heterogeneous spatial distribution of C3+ astrocytes in the spinal cord. At the behavioral level, inhibition of C3+ astrocytes led to motor function improvements, highlighting their potential as a therapeutic target for mitigating axonal damage after SCI.

Mapping the CA1 hippocampal proteome of long-term potentiation

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Synaptic plasticity, the brain's ability to adapt to new stimuli, is the neural basis of learning and memory in the hippocampus. Specifically, the most studied process of synaptic plasticity is long-term potentiation (LTP). The ability to maintain LTP is protein synthesis-dependent, some of which have been identified through pharmacological blockade studies. However, due to the complexity of memory formation and maintenance, no proteomic study has been previously performed to map protein expression in the hippocampus after LTP in vivo.
Thus, an innovative spatial proteomics study was performed to map the CA1 subfield hippocampal proteome distribution using matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry, which allows protein detection with spatial resolution directly on tissue sections. Thirty-eight adult male and female mice were implanted with stimulating electrode in CA3 and recording electrode in CA1. Two high-frequency stimulation (HFS) protocols were applied in vivo to generate early or late-LTP, respectively, in freely moving animals. Afterwards, MALDI imaging was performed in CA1 around the electrode, using a RapifleXTM MALDI TissuetyperTM TOF/TOF mass spectrometer followed by protein identification by traditional tandem mass spectrometry (MS/MS) directly on the tissue. To precisely delineate each hippocampal subfield, a Nissl staining was performed on succeeding tissue sections.
Overall, our results showed the correct in vivo LTP induction, and that 44 peptides were significantly up- or down-regulated by either HFS-protocol or sex. A significant protein-protein interaction (PPI) was observed using STRING database.
To the best of our knowledge, our work is the first that provides a mapping of the spatial distribution of the hippocampal CA1 subfield's proteome in male and female mice after in vivo LTP, which contributes to a better understanding of the neural basis of learning and memory.

Support
MCIN/AEI/10.13039/501100011033 (PID2020-115823-GBI00), JCCM and ERDF (SBPLY/21/180501/000150) and UCLM/ERDF (2022-GRIN-34354) to JDNL and LJD; MCIN/AEI/10.13039/501100011033/FEDER/EU (PID2021-122446NB-I00) to A.G. and J.M.D.-G.

Medium afterhyperpolarization potential in CA1 pyramidal neurons of developing rats

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The first weeks of postnatal development in rats are a period during which neurons and neural circuits undergo intense morphological, biochemical, and functional changes. Previous studies from the laboratory show changes in the excitability and the action potential waveform in CA1 hippocampal pyramidal neurons during the first postnatal weeks, which are associated with modifications in the expression of specific ion channels. This study analyzes the medium afterhyperpolarization (mAHP) and its regulation by time, voltage and calcium in developing CA1 pyramidal neurons. Additionally, the expression of Kv7, HCN, and SK2 channels, which have been linked to mAHP, was evaluated. Whole-cell patch-clamp in current-clamp configuration was used to record the intrinsic activity of CA1 pyramidal neurons of rats and Western blot experiments were performed to evaluate ion channel expression in the hippocampus of rats from 2 to 3 weeks. mAHP amplitude increased with the duration and the intensity of the injected current pulses, presenting a maximum amplitude near 1000 ms and with medium-intensity current pulses, respectively. With high-intensity pulses, which forced the neuron to be over-depolarized, some reduction in the mAHP amplitude was shown. We have also observed modulation of the mAHP amplitude via membrane holding potential and using different extracellular calcium concentrations or calcium chelators. We found that HCN, Kv7 and SK channels contributed to the mAHP, as the use of different channel blockers and modulators modified the mAHP amplitude. These electrophysiological findings were accompanied by changes in HCN, Kv7and SK2 channels expression. The involvement of HCN, Kv7 and SK channels in the mAHP controls CA1 pyramidal neurons excitability allowing their accommodation during repetitive firing.

This research was supported by a grant from Universidad Complutense de Madrid and Comunidad de Madrid (PR27/21-016).

Metabolic adaptations in post-phagocytic microglia: implications in glioblastoma

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Microglia are brain professional phagocytes mainly destined to the clearance of synapses, axonal debris, myelin, and apoptotic cells. Phagocytosis of apoptotic cells, known as efferocytosis, has a significant impact in our daily physiology, as it ensures tissue homeostasis and prevents inflammatory responses. However, phagocytosis is not a passive process of waste removal and microglia suffer adaptations. Using an in vivo model of “superphagocytosis” induced by low cranial irradiation (2Gy), which specifically triggered apoptosis in the neurogenic niche of the adult hippocampus, and an in vitro model of phagocytosis, we have discovered that phagocytosis results in a series of adaptations that included mitochondrial remodeling, catabolic shutdown, and transcriptional changes such as increased expression of galectin 3, and production of antioxidant polyamines. Post-phagocytic microglia suffered oxidative stress and cell death that was prevented with antioxidant N-acetylcysteine. To test whether these adaptations in microglia impaired subsequent phagocytosis, we studied their response using a brain tumor model (glioblastoma), subjected to two consecutive doses of radiotherapy. Microglia were able to overcome the stress associated with phagocytosis despite continued apoptotic challenges and maintained high phagocytosis efficiency of apoptotic tumor cells. These results suggest that the metabolic, mitochondrial, and transcriptional changes induced by phagocytosis were adaptive and intended to maintain phagocytic efficacy. In addition, the production of ROS and antioxidant polyamines by post-phagocytic microglia suggests a potential anti-tumoral effect of the phagocytic microglial metabolome that we are currently exploring. Overall, these results unlock the possibility to explore innovative therapeutic strategies for glioblastoma focused on enhancing the homeostatic functions of phagocytic microglia within the tumor microenvironment.

Metabolic flexibility of astrocytes in signaling and behavior

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Brain cell types show specific metabolic signatures that link energy conservation with cell function. For instance, the different relative dependence of astrocytes and neurons on glycolysis or oxidative phosphorylation indicates that energy efficiency is not identical across all cell types. We have shown that mitochondrial complex I (CI) and III (CIII) super-assembly differs between these cell types, being CI more tightly bound to CIII in neurons impacting on higher mitochondrial efficiency. In astrocytes, CI is more loosely bound to CIII impacting on higher mitochondrial reactive oxygen species (ROS) production. Furthermore, the metabolic reconfiguration of astrocytes against different situations impacts on the super-assembly of CI with CIII, determining energy conservation, ROS signaling, neuronal function and organismal higher-order behaviors.

Metabolic Support for Myelin Maintenance and Repair: The Role of Monocarboxylates and Their Transporters

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White matter integrity is dependent on correct myelin maintenance and axonal support by oligodendroglia. Myelin maintenance relies on constant turnover of lipids and proteins, which is dependent on the availability of carbon sources for oligodendroglial biosynthetic needs. Moreover, oligodendrocytic provision of energy fuels to the axons adds an additional metabolic demand to the propper functioning of these cells. Monocarboxylates, the family of molecules that comprises lactate, pyruvate, ketone bodies, and some short chain fatty acids are important alternative energy fuels for the cells, but their role in oligodendroglial function during white matter homeostasis and/or myelin regeneration remains underexplored. Here, I will present our data that first demonstrate the expression of monocarboxylate transporter (MCT) 2, the highest affinity MCT previously considered as predominantly neuronal, on mouse and human oligodendrocytes, as well as on mouse oligodendrocyte progenitor cells (OPCs) after demyelination, which is downregulated in patients with progressive multiple sclerosis (MS). I will then describe the effects of MCT2 loss of function on the maintenance of myelin and axonal integrity by adult oligodendrocytes, as well as on developmental myelination and myelin regeneration by OPCs.

Metaplastic priming of GIRK channels prevents hippocampal dysfunction in male and female mice of an early AD model

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Metaplasticity is "the plasticity of synaptic plasticity" meaning that prior history of synaptic activity can “prime” a synapse and interfere with subsequent induction of synaptic plasticity. In the early stages of Alzheimer's disease (AD), soluble oligomeric forms of β-amyloid peptide (oAβ) accumulate in limbic regions such as the hippocampus, leading to neuronal hyperexcitability. This hyperexcitability can influence synaptic plasticity processes, acting as an aberrant metaplastic phenomenon ultimately contributing to cognitive dysfunction. Previous studies showed that targeting ion channels involved in excitability regulation, such as G protein-gated inwardly rectifying K+ (GIRK) channels, can counteract the oAβ detrimental effects, preventing disease progression. However, further research is needed to identify therapeutic strategies directed to metaplasticity to preemptively stabilizing excitatory/inhibitory imbalances and oAβ-induced neural disturbances. Here, using both sexes of a mouse model of early AD-like amyloidosis induced by intracerebroventricular (icv.) administration of oAβ1-42, we evaluated the effects of metaplastic priming with a neuron-selective GIRK channel activator on hippocampal function. Through ex vivo slice electrophysiology, in vivo oscillatory activity recordings and spatial memory assessments, we found that oAβ disrupts synaptic metaplasticity and subsequent hippocampal oscillatory activity, and spatial memory performance in both sexes. However, metaplastic priming with the icv.-administered GIRK channel activator prior to oAβ1-42 exposure effectively prevented these deficits, preserving normal synaptic and cognitive function regardless of sex.
Our findings highlight metaplasticity modulation by selective neuronal activation of GIRK channels as a potential preventive strategy for mitigating hippocampal hyperexcitability and memory impairment in the early, asymptomatic stages of AD. By adjusting metaplasticity before pathological changes take hold, this approach may offer a promising avenue for delaying disease onset and progression. Moreover, its effectiveness in both male and female mice supports the robustness of this strategy across sexes.

Support. MCIN/AEI/10.13039/501100011033(PID2020-115823-GBI00), JCCM/ERDF(SBPLY/21/180501/000150) and UCLM/ERDF(2022-GRIN-34354) to JDNL/LJD. RD/JMF held predoctoral contracts by UCLM(2023-UNIVERS-11983) and JCCM(SBPLY/23/180502/000032).

mGluR7 modulates bidirectionally inhibitory transmission at Parvalbumin-CA1 pyramidal cell synapses

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mGluR7 is a G protein-coupled glutamate receptor located at the presynaptic active zone that modulates neurotransmitter release. Brief activation of mGluR7 activates Gi/o proteins and reduces neurotransmitter release by blocking voltage-gated Ca2+ channels. On the other hand, prolonged activation of this receptor by pharmacological or synaptic stimuli, potentiate synaptic transmission at the Schaffer Collateral–CA1 (SC-CA1) synapses by a novel signaling pathway involving the Phospholipase C activation by a pertussis toxin-resistant G protein and the diacylglycerol mediated activation of synaptic vesicle docking by Munc13-2. Since mGluR7 is also expressed on synaptic terminals of Parvalbumin expressing interneurons (PV), we decided to study its ability to bidirectionally modulate synaptic transmission at PV-CA1 synapses.
Recording IPSCs from CA1 pyramidal cells induced by SC stimulation, we have demonstrated that prolonged receptor activation causes an initial reduction followed by a potentiation of IPSC amplitude. As it occurred with excitatory synaptic transmission, the first inhibitory phase is sensitive to Gi/o protein blocker Pertussis toxin, while the potentiation phase is abolished by DAG union blocker calphostin C. This response was accompanied by an increase in the coefficient of variation and in the frequency but not in the amplitude of miniature IPSCs as well as an increase of the synaptic vesicle docking, measured by electron microscopy. We also found that endogenous activation of mGluR7 by High Frequency Stimulation on SC also potentiates GABAergic synaptic transmission and this response was absent in the presence of Group-III-mGluR antagonists, calphostin C and in slices from mGluR7KO mice. Furthermore, specific knock-down of mGluR7 at PV by Cre-loxP technology abolished pharmacological- and HFS-induced potentiation of inhibitory transmission by mGluR7.
This novel role of mGluR7 in inhibitory transmission could be decisive to understand the regulation of neuronal excitability, spatial memory and pathological conditions such as epilepsy, where mGluR7 is a target for anticonvulsant drugs.

Microglial SIRT2 deletion exacerbates Alzheimer's disease pathology in APP/PS1 mice

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Sirtuin 2 (SIRT2), a NAD+-dependent deacetylase, has been implicated in aging and neurodegenerative diseases such as Alzheimer's disease (AD). While global SIRT2 inhibition has shown promise in reducing amyloid-beta pathology and cognitive deficits in different mouse models of AD, peripheral SIRT2 inhibition has been associated with adverse effects, such as increased inflammation. This suggests that targeted inhibition of specific cellular populations within the brain may represent a more precise and effective approach for the treatment of AD. To explore this hypothesis, we generated a conditional microglial SIRT2 knockout mouse model in the context of AD, the APP/PS1- SIRT2ΔTmem119 model. Our results reveal that microglial SIRT2 reduction does not confer protective effects in the APP/PS1 model; rather, it reduced survival rates, accelerated cognitive decline, increased amyloid plaque deposition and elevated levels of pro-inflammatory cytokines at early disease stages. Moreover, in APP/PS1-SIRT2ΔTmem119 mice the percentage of Aβ plaque-coverage by microglia was decreased which could explain the increased size of amyloid plaques observed in these mice. These changes were accompanied by impaired synaptic function, alterations in dendritic spines morphology and disrupted LTP in the hippocampus. Transcriptomic analysis of isolated microglia from the hippocampus further indicates that SIRT2-deficient microglia exhibit altered expression of genes associated with aging and early AD. Pairwise analyses revealed more differentially expressed genes between APP/PS1-SIRT2ΔTmem119 and SIRT2ΔTmem119 (652 genes changed) than between Control and APP/PS1 groups (251 genes changed), suggesting a higher impact of AD pathology in a context of SIRT2 deficiency. These findings suggest that while SIRT2 inhibition in some contexts may be beneficial, targeted inhibition within microglia could accelerate AD progression, underscoring the need for cell-specific approaches when considering SIRT2 as a therapeutic target.

Microglial SIRT2 deletion impairs neuronal function

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Sirtuin 2 (SIRT2), the most expressed sirtuin in the CNS, is present in all major brain cell types. Since its pharmacological inhibition has been proposed as a potential therapeutic strategy for neurodegenerative diseases, understanding its specific role in different CNS cell types and contexts is essential.

In this study, we investigate the role of SIRT2 in microglia, key cells in brain homeostasis, in two different contexts: postnatal CNS development and adulthood. To this end, we have generated the SIRT2ΔTmem119 model, enabling microglia-specific SIRT2 deletion via tamoxifen administration at different time points: at 2 months old to study its role in adulthood and P3 to understand its function during postnatal development. A comprehensive transcriptomic, morphological, and molecular characterization has been performed in the hippocampus of control and microglial SIRT2-deficient mice at 5 months old and P25 respectively.

In adulthood, transcriptomic analysis shows altered expression of genes associated with aging and synaptic dysfunction in SIRT2-deficient microglia. This phenotype is accompanied by increased phagocytosis of synaptic elements, cognitive dysfunction, reduced neuronal activity, and impaired long-term potentiation. Similarly, at P25, transcriptomic data have revealed changes in genes related to neurogenesis and synaptic regulation. Additionally, a significant reduction in synaptic proteins PSD95 and synapsin I was found in the hippocampus. Notably, we observed increased Otx2 expression in microglia from both adult and P25 SIRT2ΔTmem119 mice. Otx2, crucial for perineuronal net (PNN) formation, restricts synaptic plasticity and defines the onset and closure of critical periods. This suggests a potential mechanism underlying the synaptic alterations in microglial SIRT2-deficient mice.

Our findings suggest a crucial role of microglial SIRT2 in CNS function, since its deficiency leads to synaptic and behavioral impairments. By linking microglial dysfunction to altered critical period dynamics and PNN development, our findings identify new therapeutic targets for cognitive impairments associated with neurodevelopmental and neurodegenerative diseases.

Molecular alterations of hippocampal glutamatergic neurons in Alzheimer's disease transgenic mice

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Altered excitatory neurotransmission in hippocampal memory circuits has been proposed to underlie memory deficits in Alzheimer's disease (AD). However, the specific cellular and molecular mechanisms by which amyloid-β (Aβ) and tau pathologies contribute to disruption of excitatory hippocampal neurons leading to memory deficits remain largely unknown.
Here, we conducted behavioral, biochemical, molecular and advanced microscopy techniques to study the effects of early Aβ and tau accumulation on hippocampal excitatory glutamatergic (CaMKIIα) neurons in transgenic mice expressing mutant human amyloid precursor protein (APP), microtubule-associated protein-Tau (MAPT) or both APP/Tau genes. In addition, we performed cell-type specific RNA-sequencing to analyze transcriptome profiles of CaMKIIα neurons in the hippocampus of APP/Tau mice.
Histopathological analyses showed accumulation of Aβ and phosphorylated Tau in CaMKIIα+ glutamatergic neurons but not inhibitory interneurons in the hippocampus of APP/Tau mice at 6 months of age. Accordingly, cell-type specific gene expression profiling in novel APP/Tau;RiboTag;CaMKIIα-Cre mice confirmed high expression of human mutant APP and MAPT genes in excitatory neurons. Male and female Tau and APP/Tau mice display hippocampal dependent memory deficits coinciding with hippocampal synaptic tau accumulation and loss of excitatory synaptic integrity. Importantly, bulk RNA-seq analyses revealed genotype and sex-dependent alterations in gene expression affecting inflammation, autophagy and myelination pathways, as well as downregulation of synaptic genes in the hippocampus of male and female Tau and APP/Tau mice. Moreover, we found transcriptional changes affecting metabolic, mRNA binding and synaptic pathways in CaMKIIα+ glutamatergic neurons of APP/Tau hippocampus.
In summary, our results show that APP/Tau transgenic mice recapitulate the neuropathological changes associated with AD, exhibiting cell-type specific alterations in glutamatergic neurons, making it a valuable tool for investigating the molecular mechanisms that contribute to the selective vulnerability of excitatory neurons in AD.

Molecular and Morphological Alterations in Oligodendrocyte Lineage Cells in Human and SNCA-OVX Mouse Models in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the gradual loss of dopamine-producing neurons in the substantia nigra pars compacta. Oligodendrocyte progenitor cells (OPCs) are found in the central nervous system (CNS) that can differentiate into oligodendrocytes, the cells responsible for myelinating axons in the CNS. Studies have suggested that OPCs may have a range of functions beyond myelination, including neuroprotection, and synaptic plasticity. The role of OPCs in PD is still unknown, however studies have shown that human single-cell transcriptome analysis from both SN and cortex have a large population of OPCs in PD subjects, and that PD genetic risk correlates better with oligodendrocyte lineage cells (OLs) than other cell types in SN.

Here we examined the expression of a snRNAseq dataset from control/PD human samples through Gene Set Enrichment Analysis. In PD samples, pathways related to oxidative stress and heat-shock proteins were found upregulated in oligodendrocytes. Interestingly, we found a significant increase in the expression of myelination-associated genes in a SNCA-overexpressing (SNCA-OVX) mouse model of PD. Therefore, we further explored the biology of cells of OL in human tissue and SNCA-OVX animals. We found an age and brain region-dependent increase in the number and size of OPCs, and also detected an increase in oligomeric α-synuclein aggregates in the processes of PDGFRα-positive cells in SNCA-OVX animals, whereas in control animals, these aggregates were preferentially observed in the soma. Additionally, the cingulate cortex of PD patients showed a larger myelinated area and a more circular morphology. In SNCA-OVX animals, we also observed an increasing trend in the myelinated area of the cortex.

Our results from both human and SNCA-OVX mice suggests that α-synuclein pathology may influence oligodendrocyte-driven myelination changes, both in SN and cortex. Further research is needed to determine the functional relevance of these findings in PD pathophysiology.

MSK1 regulates BDNF-dependent striatal neurodevelopment via modulation of MeCP2 phosphorylation

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The intricate orchestration of postnatal brain development depends, among other factors, on brain-derived neurotrophic factor (BDNF), which regulates postnatal brain development, particularly GABAergic neuron architecture, through downstream signaling pathways. In this work, we have examined the role during mouse brain development of mitogen- and stress-activated kinase 1 (MSK1), a BDNF/MAPK effector known for its governance of gene expression. We found that in early postnatal and juvenile mice, MSK1 expression is elevated in GABAergic neurons across the cortex, hippocampus, and striatum. Intriguingly, as the brain matures, MSK1 expression wanes in the cortex and hippocampus, yet increases in the striatum through adulthood, a MSK1 dichotomy hinting at region-specific roles. To probe this, we engineered Msk1IV knockout (Msk1IV KO) mice, revealing a reduction in brain weight and striatal volume, with no volumetric changes in the cortex or hippocampus. In striatal medium spiny neurons (MSNs), MSK1 deficiency uncovered a compromised dendritic landscape with reduced dendritic complexity, fewer branches, and lower dendritic spine density, contrasting with the lack of effect on cortical pyramidal neurons. Suspecting a link to methyl-CpG-binding protein 2 (MeCP2), critical for striatal morphogenesis, we explored BDNF-driven phosphorylation. We found that striatal MSNs require a novel MAPK/MSK1 pathway, contrary to the established CaMKII-dependent mechanism observed in cortical neurons, an indication of signaling divergence of BDNF between different neuronal types. Behaviorally, Msk1IV KO mice displayed disrupted sociability, anxiety, and sex-specific differences reminiscent of schizophrenia-like phenotypes. These findings establish MSK1 as a critical regulator of BDNF-MeCP2 interplay in striatal maturation, with its absence disrupting MSN morphology and behavior. This region-specific role of MSK1 suggests its dysregulation may contribute to neuropsychiatric disorders, warranting further investigation in disease models.

MSK1 regulates BDNF-dependent striatal neurodevelopment via modulation of MeCP2 phosphorylation

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The intricate orchestration of postnatal brain development hinges, among other factors, on neurotrophins, notably brain-derived neurotrophic factor (BDNF), which sculpts the architecture of GABAergic neurons through intricate unveiled signalling cascades. We investigated the role of mitogen- and stress-activated kinase 1 (MSK1), a pivotal effector within the BDNF/MAPK pathway, known for its governance of gene expression. In early postnatal and juvenile mice, we observed a striking predominance of MSK1 in GABAergic neuronal populations across the cortex, hippocampus, and striatum. Intriguingly, as the brain matures, MSK1 expression wanes in cortex and hippocampus, yet surges in the striatum through adulthood, a MSK1 dichotomy hinting at region-specific roles. To probe this, we engineered MSK1 knockout (KO) mice, revealing a profound reduction in brain weight and a markedly diminished striatum, sparing the cortex and hippocampus. Delving into striatal medium spiny neurons (MSNs), we uncovered a compromised dendritic landscape: stunted, sparsely branched neurites and a paucity of spines, contrasting with the resilience of cortical pyramidal cells. Suspecting a link to methyl-CpG-binding protein 2 (MeCP2), a linchpin in striatal morphogenesis, we explored BDNF-driven phosphorylation. While cortical MeCP2 activation aligns with the established CaMKII dependency, striatal MSNs unveiled a novel MAPK/MSK1 reliance, independent of CaMKII, an indication of signalling divergence. Behaviourally, MSK1 KO mice exhibited disrupted sociability, heightened stress and anxiety, with sex-specific distinctions reminiscent of schizophrenia. These insights position MSK1 as a critical conduit of BDNF-MeCP2 interplay and shows that dysregulation of this pathway may contribute to neuropsychiatric phenotypes, warranting further exploration in disease models.

Myelin boosts oligodendroglia lineage proliferation and maturation

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Oligodendrocytes are the myelinating cells of the CNS. However, their functions extend beyond the synthesis of the myelin sheath, providing energy substrates to axons to support action potential propagation or acquiring immune properties in pathological states. In this study, we investigated whether oligodendrocytes can internalize and metabolize myelin components to promote their survival and differentiation. To this end, we used Alexa488-labeled myelin and fatty acids in cell cultures and in vivo models, combining viability assays with confocal and electron microscopy.
Our results showed that oligodendrocyte cultures internalize exogenous myelin and oleate in their proximal processes, which accumulate in lipid droplets over time. Notably, we found that myelin enhances oligodendrocyte survival and differentiation, primarily by stimulating proliferation. Myelin-induced lineage progression also results in an increased myelination on nanofiber coverslips. Furthermore, a mixture of four fatty acids replicated the higher cell survival, suggesting that myelin lipids might be responsible for this effect.
Stereotaxic injections of myelin into the cortex of adult mice revealed myelin debris inside microglia and oligodendroglia, confirming the internalization previously found in vitro. Similarly, cerebroventricular injections of oleate in fasted mice showed widespread incorporation of the fatty acid into various cell types, including mature and progenitor oligodendrocytes.
To validate the proliferative effect of myelin observed in vitro, we performed cerebroventricular injections of myelin into 3dpf zebrafish. In vivo imaging of the spinal cord after 24 hours revealed an increase in the number of ventral oligodendrocytes, which further supports the idea that myelin debris causes a boost in proliferation. Additionally, we also observed myelin debris inside microglia and mature oligodendrocytes.
Together, these findings demonstrate that oligodendrocytes internalize myelin components, which fuel their proliferation, differentiation, and survival.
Supported by Spanish Ministry of Sciences (PID2022-143020OB-I00, PID2022-138276OB-I00), CIBERNED (CB06/05/0076), Basque Government (IT1551-22). C.P.M. holds a fellowship from the Spanish Ministry of Sciences.

Myelin plasticity mediated by NMDA receptors containing GluN3A subunits in oligodendrocyte progenitor cells

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Myelin plasticity mediated by NMDA receptors containing GluN3A subunits in oligodendrocyte progenitor cells

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Work over the last decade demonstrates that the myelin sheaths enwrapping axons, classically seen as static insulators, are highly dynamic and respond to axonal activity and experience. This phenomenon, called “myelin plasticity”, goes beyond postnatal stages into adulthood and plays a key role in remodeling neuronal circuits to support adaptive behavior or functional recovery upon injury. Yet the mechanisms are unknown. Here we show that NMDA receptors containing GluN3A subunits are expressed by oligodendrocyte progenitor cells (OPCs) and essentially required for activity-dependent myelin plasticity. Specifically, a functional analysis of constitutive or OPC-restricted Grin3a knockout mice demonstrated that OPCs that lack GluN3A fail to differentiate in response to chemo-genetic stimulation of callosal axons. Similar deficits were observed in conditional Grin3a knockout mice where: 1) selective OPC deletion was driven by Sox-Cre or inducible PDGFRα-CreERT2 lines; 2) OPC responses were triggered using natural paradigms that elicit myelin plasticity, including chronic physical exercise on a treadmill or fine motor training in the Erasmus Ladder. At the behavioral level, OPC-selective GluN3A deletion from OPCs impaired motor performance upon challenges. Our study identifies the first known molecular sensor for myelin plasticity, providing an opportunity to develop strategies to boost myelin plasticity based on re-expressing GluN3A or modulating downstream activity-induced signaling pathways.

Neuronal ensembles encoding sensory behavior are defined by astrocyte excitability

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How the primary somatosensory cortex (S1) discriminates and processes simultaneous information from four different modalities (touch, temperature, pain and proprioception) remains a longstanding question in neuroscience. Here, we hypothesize that cortical astrocytes fine-tune the spatially and temporally organized activity of neuronal ensembles encoding sensory modalities, thereby influencing behavior. To investigate this, we employed in vivo chemogenetic manipulation of astrocytes and behavioral assessment combined with microendoscopic calcium imaging (miniscope), to record the simultaneous activation of neuronal sensory ensembles across S1 layers. Our in vivo imaging data, obtained from freely moving animals, showed that peripheral tactile and thermal stimulation activate distinct neuronal ensembles across the cortical layers, with a small subset of multimodal neurons participating in both tactile and thermal ensembles. Furthermore, in vivo modulation of astrocyte Ca2+ activity using Gq-DREADDs enhanced the number of multimodal neurons, indicating increased flexibility of single neurons to shift from ensemble to ensemble in the presence of a disrupted astrocytic network. However, rather than being beneficial, the induced multimodality impaired texture and temperature discrimination in behavior tests, suggesting that neuronal ensembles recruited under disrupted astrocyte activity are broadly tuned and less specialized to discriminate the arriving inputs. Thus, our results demonstrate that S1 ensures the activation of the appropriate neuronal ensembles to process sensory modalities, thereby enabling accurate behavioral responses.

Neurons and glia utilize myelin-derived fatty acids as an energy source

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The brain, despite accounting for only 2% of body weight, consumes 20% of oxygen and 25% of glucose, underscoring its high metabolic demand. Maintaining energy homeostasis is crucial for CNS function, and impaired metabolic flexibility is associated with neurodegeneration. Long-chain fatty acids, which generate high ATP levels upon oxidation, require mitochondrial import via carnitine palmitoyltransferase 1A (CPT1A). Here, we investigated whether CNS cells utilize myelin-derived fatty acids (mFAs) as an alternative energy source under glucose deprivation.
Western blot analysis confirmed CPT1A expression across CNS cells, highlighting their capacity for fatty acid β-oxidation. Rhodamine 123 dequenching analysis in neurons revealed that mitochondrial membrane potential, which is compromised under glucose deprivation, is restored in the presence of mFAs (oleate, palmitate, arachidonic acid, and linoleic acid), supporting their role in maintaining neuronal energy metabolism during metabolic stress. Additionally, Seahorse flux analysis of astrocytes demonstrated that mFAs enhance maximal respiration under glucose deprivation—an effect abolished by pharmacological inhibition of CPT1A—indicating that mFA β-oxidation is actively utilized for energy production. Finally, in an ex vivo mouse optic nerve model, mFAs significantly improved the recovery of compound action potentials following glucose deprivation. Collectively, these findings demonstrate that mFAs serve as an energy source to support neurons and astrocytes when glucose is scarce.
Supported by Spanish Ministry of Sciences (PID2022-143020OB-I00), CIBERNED (CB06/05/0076) and Basque Government (IT1551-22). E.L.-M. and A. P. holds fellowships from Basque Government and the Spanish Ministry of Sciences, respectively.

Novel Dysfunctional Connectivity in the Prefrontal Cortex of a Mouse Model of Compulsive Behavior

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Obsessive-Compulsive Disorder (OCD), affecting 2-3% of the global population, is linked to corticostriatal dysfunction. In Sapap3 knockout (KO) mice, a model of compulsive behavior, hyperactivity is observed in the secondary motor area (M2), similar to hyperactivity in the supplementary motor area of OCD patients. Cortical disinhibition due to GABAergic deficits is proposed to contribute to OCD pathogenesis.

In this study, we examine the role of parvalbumin-positive (PV+) interneurons in the M2 cortical GABAergic circuitry of Sapap3 KO mice, using in vitro and in vivo methods, to understand their contribution to compulsive behavior.

Sapap3 KO mice showed fewer PV+ interneurons in M2, while the remaining ones displayed unexpected hyperexcitability, as determined by intrinsic property analysis. Subsequent analysis of spontaneous excitatory and inhibitory synaptic inputs onto PV+ interneurons revealed no significant differences. Interestingly, retrograde tracing identified a cortical pathway connecting the lateral orbitofrontal cortex (lOFC) and M2. Employing optogenetic stimulation in brain slices, we discovered a significant increase in NMDAR-mediated glutamatergic signaling from lOFC to PV+ interneurons in M2 of the Sapap3 KO mice.

We performed in vivo two-photon calcium imaging in awake animals to examine PV+ cell activity in M2 during compulsive grooming, demonstrating a positive correlation between increased PV+ activity and grooming bouts. To further understand this behavior, future studies will delineate the contribution of the lOFC-M2 pathway. Ultimately, we will manipulate PV+ interneuron activity in awake mice to assess its causal role in compulsive grooming.

We are grateful to C. Cabrera Romero for excellent technical assistance. Supported by: RyC-2016-19906, PRE2019-087729 (MCI/AEI/FSE, UE), VI PPIT-US, US-1264432 (US/JUNTA/FEDER, UE), PREDOC_00594 (Junta de Andalucía) and PID2021-123840NB-I00 (MCIN/AEI/10.13039/501100011033/FEDER, UE).

Optimizing dopamine storage within secretory vesicles of dopamine-differentiated neuroblastoma cell line BE(2)-M17

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Neurosecretion is a finely regulated process essential for neuronal communication, relying on the precise release of neurotransmitters via exocytosis. Dopamine signaling, depends on efficient storage within secretory vesicles to ensure rapid and reliable synaptic transmission. Disruptions in vesicular dopamine storage can alter signal strength and duration, contributing to disorders like Parkinson's disease.

Dopamine storage into secretory vesicles is a tightly regulated process, primarily controlled by VMAT-2. Furthermore, experimental evidence shows that supplementation with L-DOPA or fluorescent false neurotransmitters enhances intravesicular dopamine storage. However, a deeper understanding of the factors regulating storage within these vesicles remains crucial. Identifying new regulatory factors that promote vesicular dopamine storage is essential for optimizing dopamine availability at synapses, ultimately improving the effectiveness of therapeutic strategies for dopamine-related disorders.

BE(2)-M17 cells serve as a valuable dopaminergic neuronal model for studying dopamine storage mechanisms. Derived from neuroblastoma, this cell line can differentiate into dopamine-producing neurons, making it an ideal system for exploring molecular and pharmacological strategies aimed at optimizing dopamine accumulation within secretory vesicles. Unlike PC12 cells, which originate from chromaffin tissue, BE(2)-M17 cells more closely resemble human dopaminergic neurons, offering a more accurate model for screening compounds that enhance vesicular dopamine storage and better reflect the complexities of human dopamine regulation.

This study aims to explore pharmacological strategies to optimize dopamine sequestration within vesicles, with a particular focus on enhancing storage capacity. The ultimate goal is to establish a model cell line that improves dopamine availability at synapses, ensuring more efficient neurotransmission. Using amperometry, an advanced electrochemical technique, we will assess dopamine levels and gain valuable insights into the efficiency of both storage and release processes. By enhancing dopamine storage efficiency, we seek to deepen our understanding of dopamine homeostasis and provide new insights for developing more effective therapeutic interventions for dopamine-related disorders, such as Parkinson.

OVEREXPRESSION OF STMN2, A MICROTUBULE DESTABILIZING PROTEIN, ENHANCES PRESYNAPTIC NEUROTRANSMITTER RELEASE AND POSTSYNAPTIC RESPONSE

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Stathmin-2 (STMN2) is a microtubule-binding protein associated to vesicles that promotes microtubule (MT) instability. It is well established that STMN2 is upregulated in neurons after injury, however, the physiological relevance and the mechanisms activated by a transient increase of STMN2 remain unknown. An upregulation of STMN2 was replicated on autaptic hippocampal neuronal cultures by infection with lentiviruses containing STMN2-GFP, and neurotransmission was recorded by the whole cell patch-clamp technique. We discovered that the overexpression of STMN2 modified presynaptic properties by selectively enhancing spontaneous neurotransmitter release, which increased from 0.9 s^{-1} to 3.6 s^{-1} (n=25). Since the activity of STMN2 is negatively regulated by phosphorylation, we tested the effect of Okadaic Acid, a non-specific phosphorylating agent, and Anisomycin, an activator of JNK, which is hypothesized to phosphorylate STMN2. Both drugs decreased spontaneous neurotransmitter release by 50% (n=5). We also demonstrated that the effect of STMN2 in neurotransmission was reverted by MT stabilization in a concentration dependent manner by ATAT1, a MT acetylating enzyme, reducing spontaneous release frequency by 50% (n=10). We observed that STMN2 had also a postsynaptic effect, as the amplitude of mEPSCs increased by 30% when overexpressed (n=50). Non-stationary noise analysis applied to WT neurons revealed that approximately 35 glutamate receptors with a single channel conductance of 8 pS contribute to a mEPSC (n=3). In neurons overexpressing STMN2, the number of glutamate receptors doubled, while the single channel conductance was maintained to 6 pS (n=3).
Considering the fundamental role of spontaneous neurotransmitter release in the assembly of developing neuronal circuits, we suggest a link among STMN2, MT dynamics and spontaneous neurotransmission that promotes the formation of synaptic contacts. Our findings could be key to explain the increase of STMN2 levels occurring upon neuronal injury and acknowledge its relevance in the homeostasis of synaptic functions.

Plastic Properties of Hypothalamic Oxytocinergic Neurons

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Oxytocinergic neurons, responsible for producing and releasing the hormone oxytocin (OXT), are essential for regulating social behavior, attachment, and stress responses. Recent research indicates that neurons within the paraventricular nucleus of the hypothalamus (PVN) exhibit considerable plasticity, enabling them to modify their connectivity in response to environmental changes. Understanding the properties and mechanisms that regulate this plasticity is crucial for examining the role of the oxytocinergic system in both normal physiological functions and in various pathological states.

To investigate the basic rules governing plasticity of PVN oxytocinergic neurons, we conducted visually-guided electrophysiological recordings to assess how different stimulation protocols—known to induce various forms of synaptic plasticity in other brain regions—affect these neurons. Unexpectedly, while high-frequency stimulation (300 pulses at 100 Hz), typically associated with long-term potentiation in the hippocampus, failed to induce synaptic changes, a brief theta burst stimulation consistently triggered moderate long-term depression. Additionally, we manipulated OXT signaling either by the application of a specific OXT receptor antagonist or by reduction of OXT release using siRNA-dependent gene silencing. Our results strongly suggest a critical role of OXT signaling in baseline synaptic properties but also in modulating synaptic modifications of PVN oxytocinergic neurons.

Our findings reveal that long-term depression is a dominant form of plasticity in PVN oxytocinergic neurons and underscore the importance of basal OXT signaling in facilitating synaptic transmission in this region. This, in turn, may be linked to the regulation of various homeostatic functions and behaviors such as social interaction. These results provide a foundation for further investigation into how imbalances in OXT may affect neuronal physiology and social behavior.

Plasticity of neuronal networks and GABAergic dynamics of the primary somatosensor cortex after a spinal cord injury

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A complete thoracic spinal cord injury (SCI) induces physiological changes in the primary somatosensory cortex of the hindlimbs (S1HL), characterized by neuronal network hyperexcitability. Additionally, we have observed a decrease in GAD67 labeling intensity in the infragranular layers of S1HL in animals with chronic SCI. However, how this reduction in GAD67 is related to the altered inhibitory function of GABAergic neurons across cortical layers after SCI remains unknown.
This study has two main objectives: to characterize the physiological effects of chronic SCI on different GABAergic neuronal populations in each cortical layer and to investigate the functional relationship between cortical hyperexcitability and GABAergic activity following SCI.
To achieve this, we used a Cre line mice that express the calcium genetic probe GCaMP6f, to monitor intracellular calcium activity, in the GABAergic populations of each cortical layer within S1HL. GABAergic activity was analyzed using ex vivo epifluorescence microscopy in coronal slices of S1HL from control animals and 15 or 30 days after a thoracic SCI. Additionally, we assessed the electrophysiological activity of all six cortical layers in animals before and after SCI, to quantify the frequency ranges of spontaneous activity and the magnitude of evoked responses in awake, head-fixed animals.
Our results reveal that, in SCI animals, the activity profile of GABAergic neuronal populations differs across cortical layers, with supragranular layers being more active than infragranular layers. Furthermore, in vivo recordings show that SCI increases high-frequency oscillations and produces a higher neuronal firing rates in infragranular layers.
In conclusion, SCI differentially alters GABAergic activity across S1HL layers, which could explain the increased infragranular excitability observed in vivo. Our results contribute to explain changes in cortical networks after SCI and its relation with associated pathologies.

Reducing P11 expression affects synaptic vesicles dynamics at excitatory inputs on rat pups hypoglossal motoneurons

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P11 (S100A10) is known by its role in regulation of trafficking and membrane expression of several receptors and ion channels. Recently, we have showed that P11 also controls neurotransmission at excitatory synapses of hypoglossal motoneurons (HMNs), presumably by interacting with MUNC13-1, a presynaptic active zone protein which mediates synaptic vesicle (SV) priming. Here, we further investigated a feasible role of P11 in the control of SVs dynamics. Thus, P11-induced down-regulation (-46.4 ± 1.7%, P7) after microinjection in the 4th ventricle of a small-interfering RNA directed against mRNAp11 (siRNAp11; 5 µg/5 µl, P5) resulted in depression of excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation of the ventrolateral reticular formation (VLRF) in patch-clamped HMNs (P7-P9), as compared with a non-interfering siRNA (cRNA: 0.57 ± 0.07 nA; siRNAp11: 0.24 ± 0.03 nA). Frequency, but not amplitude, of spontaneously occurring EPSCs was reduced while EPSC facilitation during high frequency stimulation (HFS, @20 Hz, 1-s) was increased by P11 down-expression, supporting thus a presynaptic mechanism of action. Our results also reveal a strong decline of both, the synchronous and asynchronous components of neurotransmitter release during long-lasting HFS protocol (@60 Hz, 10-s) under siRNAp11 treatment. Offline analysis evidenced a reduction of the readily-releasable pool (cRNA: 0.037 ± 0.005 nC; siRNAp11: 0.011 ± 0.003 nC) and replenishment rate (cRNA: 0.493 ± 0.082 nC/AP; siRNAp11: 0.151 ± 0.025 nC/AP) of SVs without affectation of release probability in the siRNAp11 condition. Finally, time course analysis of post-HFS EPSC charge transfer showed a slower and incomplete recovery under P11 interference. Altogether, these outcomes support a key role of P11 in the management of SVs dynamics at excitatory synapses.

Funding: Grant PID2023-152180NB-I00 funded by MICIU/AEI/10.13039/501100011033 and by “ERDF/EU”.

Regulating fear conditioning in wild-type and FMR1KO mice

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Autism is a neurological disorder characterized by anxiety-like behavior, deficits in associative learning, and in social interaction. The FMR1KO mice is the most studied model of monogenic autism. Fear conditioning is a form of associative learning and memory that has been the focus of numerous studies centered on the neuronal functionality and connectivity of the brain regions involved in this learning process. However, the regulation of this associative learning process by neuromodulators is largely unexplored. The aim of this research was to test the effects of insulin-like growth factor-1 (IGF-1) and astrocyte activation as neuromodulators to regulate associative learning behavior in wild-type mice and FMR1KO mice.
Astrocytes modulate synaptic transmission, and IGF-1 plays a key role in synaptic plasticity, spatial learning, and anxiety-like behavioral processes. We have found that optogenetic activation of astrocytes in the prelimbic area of the medial prefrontal cortex, a brain area involved in fear conditioning, increases fear expression in wild-type mice. This increase in fear levels correlates with an increase in the AMPA/NMDA ratio of the prelimbic layer 5 pyramidal neurons, suggesting an increase in synaptic efficacy as the cellular mechanism of this effect. Interestingly, although IGF-1 decreases fear levels by favoring fear extinction in the wild-type mice, we found that IGF-1 increases fear expression in FMR1KO mice. These results suggest that the effect of IGF-1 is different in wild-type and FMR1KO mice and that astrocytes contribute to the associative learning process.

REGULATION AND FUNCTION OF THE GLUCOSE TRANSPORTER GLUT4 IN THE HIPPOCAMPUS

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Glucose is the main energy source of the brain; however, how glucose is metabolized by different brain cells is still under debate. Neurons and astrocytes express different glucose transporters (GLUTs), but only neurons express the insulin-sensitive glucose transporter GLUT4, whose surface expression is under tight regulation in adipocytes and myocytes. In neurons, electrical stimulation has been shown to induce GLUT4 membrane translocation, at least in presynaptic terminals. To get a better understanding of brain metabolism, our group aims to characterize the function and regulation of GLUT4 in postsynaptic compartments of hippocampal neurons. Performing immunohistochemistry assays in organotypic hippocampal slice cultures, we first found that the surface to total ratio of recombinant GFP-tagged GLUT4 is higher in spines than in dendrites under basal conditions. Interestingly, we found that surface GFP-GLUT4 undergoes continuous recycling in a glucose- and sex-dependent manner. Then, to understand the functional role of GLUT4 in synaptic transmission and plasticity, we performed electrophysiological field recordings in acute hippocampal slices using the GLUT4-specific inhibitor indinavir. We found that acute application of indinavir decreases basal transmission at CA3-to-CA1 synapses. We are now investigating whether this effect is mediated by presynaptic or postsynaptic factors. Overall, this research points to GLUT4 as a neuronal glucose provider needed for synaptic activity and may reveal differential regulation depending on the sex.

Retinal Müller Glia Mechanosensitivity: How Different Mechanical Cues Modulate Müller Glia Response

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In many retinal pathologies like glaucoma, retinal detachment or epiretinal membranes, mechanical factors —including extracellular matrix (ECM) stiffness, intraocular pressure, and shear forces at the vitreoretinal interface— are altered, contributing to fibrotic processes. Müller glia (MG) are mechanosensitive cells exclusive to the retina. This study analyzed how different mechanical cues influence MG survival, cell area, and ECM deposition in vitro.
Primary rat MG were subjected to three different mechanical forces: 1) Stiffness, culturing the cells on polyacrylamide (PAA) gels (10 and 100 kPa) and glass (GPa) as control; 2) high pressure (HP) for 72 hours, achieved using a specific chamber; and 3) shear stress, by seeding the cells in chambers exposed to a 10^-3 dyn/cm² flow rate for 24 hours. MG were labeled for morphology (vimentin) and ECM deposition (collagen I, IV, fibronectin). Cells were quantified and cell area and the intensity of ECM deposition were measured with ImageJ.
MG survival was significantly reduced on softer PAA gels (31.08±4.81% on 100 kPa, 3.13±0.57% on 10 kPa) compared to glass (considered 100%). Similarly, cell area decreased on softer substrates. ECM deposition was stiffness-dependent, with MG depositing more collagen I (45.79±1.09%), collagen IV (63.89±2.44%), and fibronectin (38.58±1.14%) on stiffer gels than on softer ones (12.70±0.47%, 21.90±1.48%, and 20.62±0.64%, respectively). HP further enhanced ECM deposition but reduced overall cell survival. Flow-induced shear stress promoted MG survival (126.28±4.93%) compared to static conditions (considered 100%) but decreased cell area. Fibronectin deposition increased under flow (190.00±8.03%); however, although collagen I and IV intracellular expression increased (145.31±1.46% and 191.81±3.03%, respectively) there was no extracellular deposition under any condition.
This study highlights the mechanosensitive nature of MG, showing that different mechanical cues distinctly affect survival, morphology, and ECM deposition. These findings emphasize the role of the biomechanical environment in retinal fibrotic processes and their significance in retinal pathophysiology.

Role of Galectin-3 in the Modulation of Hippocampal Neurogenesis

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Hippocampal neurogenesis is essential for learning, memory, and cognitive flexibility. Emerging evidence highlights the pivotal role of microglia, the brain's resident immune cells, in regulating this process. Galectin-3 (Gal-3), a β-galactoside-binding lectin predominantly expressed by microglia, has been implicated in neuroinflammation and neural plasticity. However, its specific role in hippocampal neurogenesis remains unclear. This study aims to investigate the impact of microglial Gal-3 on adult hippocampal neurogenesis and elucidate the underlying mechanisms. We hypothesize that Gal-3 modulates the proliferation, survival, and differentiation of neural progenitor cells within the dentate gyrus. To test this, we employ a multifaceted approach combining in vitro and in vivo experiments using Gal-3 knockout models. By analyzing neurogenesis markers, microglial activation states, and the neurogenic niche, we seek to unravel the intricate interactions between microglia and neural progenitors. This research could serve as a starting point for developing targeted therapies to promote hippocampal neurogenesis in conditions related to cognitive decline and reduced neuroplasticity.

Role of NCAM2 in cell-matrix and cell-cell interactions in synaptic plasticity

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Synaptic plasticity is considered to be the primary mechanism driving neural adaptability and is crucial for learning, memory, and cognitive functions throughout life. At the molecular level, extracellular matrix molecules, such as Reelin, and transmembrane cell adhesion molecules (CAMs) play an important role in regulating and adapting synaptic changes, with important implications in pathology.
CAMs have emerged as key elements in the regulation of adult neuronal plasticity, playing prominent roles in controlling synapse formation and maintenance. Neural Cell Adhesion Molecule 2 (NCAM2) is a cell adhesion molecule specifically expressed in brain that we found crucial for proper neuronal morphogenesis and cortex formation. In our studies, we characterized the interactome of NCAM2, identifying over 100 proteins that interact with NCAM2. Furthermore, our proteomic data, analyzed using bioinformatic tools, revealed significant enrichments in gene ontology terms and cellular pathways linked to the cytoskeleton and important neural cognitive functions, such as synapse formation and synapse maintenance.
Here we show that in the adult brain, NCAM2 plays a crucial role in proper synaptic plasticity. Our results indicate that NCAM2 is involved in synapse formation and stability, and that physiological levels of NCAM2 are essential for synapse maintenance in the adult nervous system. Moreover, depletion of NCAM2 leads to dendritic spine constriction and a reduction in spine density. Conversely, elevated levels of the gene may enhance contact stabilization by increasing dendritic spine size.
Adhesion molecules and components of the extracelular environment has been linked to neuroprotective and neurodegenerative mechanisms in pathology, including Alzheimer's disease. Therefore, the findings from this study on NCAM2 deepens our understanding of the synaptic deficits associated with these conditions and pave the way for promising new research directions.

Selective GIRK1 Deletion in Lhx6 Interneurons Restores Network Function and Memory Deficits in a Mouse Model of Alzheimer's Disease

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Neuronal hyperexcitability is an early feature of Alzheimer's disease (AD) that contributes to network dysfunction. GIRK channels, key regulators of neuronal excitability, have been implicated in this process. Lhx6-expressing interneurons (PV+SST) play a crucial role in maintaining network synchrony, and their dysfunction is associated with hypersynchrony, spontaneous epileptiform discharges, and reduced gamma oscillations in the J20 mouse model of AD. Here, we investigated the role of GIRK channels in Lhx6 interneuron activity and their contribution to AD-related hyperexcitability.
RNA-sequencing analysis revealed an upregulation of the GIRK1 subunit in Lhx6 interneurons of the parietal cortex in J20 mice, suggesting a role in network dysfunction. To determine whether selective GIRK1 deletion in Lhx6 interneurons could restore excitability and associated network and cognitive functions, we generated bigenic Lhx6-Cre;GIRK1-flox;J20 mice, enabling targeted GIRK1 deletion in these interneurons.
We assessed ex vivo oscillatory activity using optogenetic stimulation at different frequencies, hippocampal excitability through input/output (I/O) curves, and synaptic plasticity via long-term potentiation (LTP) at the CA3-CA1 synapse. Additionally, in vivo electrophysiological recordings were performed to quantify spike number, and cognitive function was evaluated using contextual fear conditioning.
Our results show that selective GIRK1 deletion in Lhx6 interneurons facilitated gamma oscillation induction. In the J20 AD model, this approach also counteracted hyperexcitability and restored synaptic plasticity. Furthermore, in bigenic mice, spike number was decreased, and memory deficits in the fear conditioning test were counteracted, indicating that reducing GIRK activity in Lhx6 interneurons via GIRK1 deletion is sufficient to restore cognitive function.
These findings establish a critical role for GIRK1 channels in Lhx6 interneurons in AD-related network dysfunction. Targeting GIRK1 in these cells represents a promising therapeutic strategy to mitigate hyperexcitability and cognitive impairment in AD.

MCIN/AEI/10.13039/501100011033 (PID2020-115823-GBI00), JCCM and ERDF (SBPLY/21/180501/000150) and UCLM/ERDF (2022-GRIN-34354) to JDNL and LJD. MCIN/AEI/10.13039/501100011033/FEDER/EU. JMF, JCCM predoctoral contract (SBPLY/23/180502/000032).

SEX-RELATED DIFFERENCES IN CAFFEINE'S EFFECT ON SPONTANEOUS ACh RELEASE IN MICE

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Caffeine, a widely consumed stimulant, is structurally similar to adenosine, a neurotransmitter that binds to purinergic receptors in the brain. Peripherally, caffeine enhances calcium release in muscle cells, improving muscle contraction. This study aims to determine whether caffeine increases ACh release, potentially linking this effect to its ergogenic properties and the risk of fatal overdose.

The methodology includes intracellular recordings and endplate noise recordings in mice. Intracellular recordings were obtained from the diaphragm to measure the amplitudes and frequencies of miniature endplate potentials (mEPPs), assessing ACh release. Endplate noise recordings were conducted on the gastrocnemius muscle. Mice were exposed to varying caffeine concentrations.

The results indicate that acute caffeine exposure modulates ACh release in a concentration-dependent manner. In young male mice, the lowest caffeine concentration (60 µM) doubled mEPP release, with this effect persisting at 120 µM and 1 mM. However, at 5 mM, ACh release was completely inhibited. Young female mice exhibited a similar pattern of increased mEPP release at lower concentrations, though the inhibition at 5 mM was only partial. In aged mice, caffeine also enhanced mEPP release, with the most pronounced increase observed in aged females at 1 mM. Additional experiments with CADO (an adenosine analog) and atropine were conducted to further investigate the mechanisms underlying caffeine's effects on ACh release.

In summary, caffeine appears to enhance ACh release at lower concentrations but may inhibit it at higher concentrations, raising important questions about its ergogenic effects and potential overdose risks.

Structural alterations of pyramidal cells in Alzheimer´s disease individuals

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Alzheimer disease (AD) is a neurodegenerative disorder whose principal hallmarks are the presence of amyloid plaques and the intracellular accumulations of phosphorylated tau protein. Previous studies in our laboratory have shown that presence of phosphorylated tau (PTau+) gives rise to a progressive loss of dendritic spines in individuals with AD. In the present study, we further analyze in 3D the structure of dendritic and local axonal arbors of pyramidal neurons in the hippocampal formation of AD individuals. By using intracellular injections of Lucifer Yellow in fixed tissue, we show that both dendritic and axonal diameters, but not dendritic segment length, are reduced in PTau+ pyramidal neurons during the early stages of the disease. These results show that intracellular accumulations of phosphorylated tau alters selectively and progressively the architecture of human pyramidal cells, which would imply particular alterations in the functional properties of these pyramidal cells.

Structural plasticity of dendritic spines during mGluR-induced neuronal activity-dependent long-term depression

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Learning and memory are explained at the molecular level through the plastic properties of synapses. Most excitatory synapses are located on dendritic spines, which can undergo both functional and structural modifications. Typically, functional and structural plasticity are correlated: spines enlarge during long-term potentiation (LTP) and shrink during long-term depression (LTD). This holds true when LTD is induced via the NMDA type of glutamate receptors (NMDARs). However, when LTD is triggered through metabotropic glutamate receptors (mGluRs), spines do not shrink. The mechanisms underlying this difference are unknown.

To investigate how the interaction between NMDARs and mGluRs regulates spine plasticity, we used organotypic hippocampal slice cultures from postnatal rats. Slices were biolistically transfected with a fluorescent volume marker (DsRed2) and a Ca^{2+} sensor (GCaMP6), and spine morphology dynamics after chemical LTD induction were assessed via time-lapse live confocal imaging.

We found that the levels of neuronal activity in the hippocampal circuit and the concentration of divalent cations strongly influence the fate of spine morphology after DHPG-induced mGluR-LTD. Elevated Ca^{2+} favored spine enlargement, while reduced Ca^{2+} enhanced spine shrinkage. At moderate Ca^{2+} levels, spines remained unaltered, but lowering Mg^{2+} levels induced spine shrinkage. At low Ca^{2+} concentration (1 mM), spine shrinkage occurred regardless of Mg^{2+} concentration, but only if the frequency of neuronal activity oscillations in the hippocampal circuit was high. Mechanistically, at 1 mM Mg^{2+}, spine shrinkage required NMDAR activation but was protein synthesis-independent, whereas at 0.2 mM Mg^{2+}, spine shrinkage was NMDAR-independent but required de novo protein synthesis.

These findings reveal a novel form of spine structural plasticity -mGluR-induced neuronal activity-dependent LTD (MIND-LTD)- and suggest that mGluRs may participate in a metaplasticity mechanism, acting as sensors of synchronous activity to shape the long-term consolidation of dendritic spine structural plasticity.

Synapse-specific properties are defined by the distinct molecular identity of the neurotransmitter release machinery

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Neurons can establish diverse synaptic contacts that vary in their properties during ongoing neurotransmission. We hypothesize that synapse heterogeneity across the brain relies on a distinct presynaptic molecular composition, which imparts unique properties to each synapse, being crucial for key brain functions such as motor control or learning.
Traditionally, it was thought that the synaptic vesicle-(SV) release machinery was composed of a standard set of 'canonical' proteins that exhibited minimal variation between synapses. However, this is at odds with the fact that synaptic strength and short-term plasticity properties closely associated with the presynaptic terminal differ between synapses when exposed to identical activity patterns.
To address whether presynaptic protein expression remains consistent across synapses, we performed confocal imaging on mouse hippocampal and cerebellar slices, immunolabeled against key constituents of the SV release machinery, including Munc13-1, Munc13-2, Munc18-1, syntaxin-1A, syntaxin-1B, synaptotamin-1, synaptotagmin-7, complexin-1, complexin-2, alfa-synuclein and beta-synuclein. Post-hoc analysis of the expression of these proteins was conducted on excitatory and inhibitory pre-synapses, dissected from specific subregions. Our results revealed that protein expression is not uniform across synapses, with certain isoforms being more highly expressed in specific synapses than others that reside within the same region.
We corroborated our imaging findings with patch-clamp data from synapses enriched with complexin-1 and synaptotagmin-7, using single and double knockout mouse models. Our results demonstrated that the expression of these two proteins influenced synaptic properties in opposing directions. Complexin-1-deficient synapses exhibited reduced neurotransmitter release and increased short-term facilitation, while synaptotagmin-7-deficient synapses displayed synapse strengthening and short-term depression. Strikingly, double-knock-out synapses showed invariance in short-term plasticity, indicating that the opposing effects of these two proteins might balance each other.
Our research confirms that synaptic behavior is highly sensitive to the expression of individual presynaptic proteins. Mutations in these factors can lead to synapse-specific synaptopathies, eventually distorting brain microcircuit functions.

Synaptophysin family: Bi-directional regulators of synaptic vesicle release probability

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Synaptophysin family proteins are among the most heavily expressed synaptic vesicle proteins in possibly every synapse throughout the animal kingdom. The function has been a mystery until recently. Other heavily expressed synaptic vesicle proteins are required for even rudimentary synaptic transmission, but synaptophysin proteins are not. Instead, knocking out the entire family enhanced high fidelity synaptic transmission 2-fold by increasing probability of release. The four family members expressed in mammalian synapses are synaptophysin (Syp), synaptoporin (Synpr), and two synaptogyrins (Syngr 1 and Syngr 3). Adding Syp or Syngr 3 back to the knockout attenuated synaptic transmission to control levels, suggesting the two are endogenous negative regulators of synaptic vesicle exocytosis. We now use optical imaging and patch-clamp electrophysiology to show that Synpr has the opposite effect; adding Synpr back to the knockout increases synaptic transmission an additional 2-fold. The result: strengthens the hypothesis that endogenous synaptophysin family members regulate the mechanism that catalyzes synaptic vesicle exocytosis by addressing a key caveat; expands the range of regulation to more than 4-fold; and suggests that the C-terminal tail determines whether family members are positive or negative regulators. Finally, positive regulation by Synpr provides a unique tool for probing a poorly understood limit on the efficiency of the synaptic vesicle exocytosis that may be involved in learning and memory

Targeting Microglial ADP Receptors to Modulate Alzheimer's Disease Progression: Insights from a Novel APP/Tau Mouse Model

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Microglial cells are essential for maintaining brain homeostasis, contributing to synaptic plasticity and modulating neuronal activity through their interaction with neurons. Disruptions in microglia-neuron communication have been linked to neurodegenerative diseases, such as Alzheimer's disease (AD). This research aimed to investigate the role of microglial ADP receptors - activated by ATP released from distressed neurons - in AD pathogenesis, examining their potential effects on neuronal activity and disease progression.

To determine the role of microglial ADP receptor activity on AD progression, we used a novel APP/Tau transgenic mouse model that recapitulates key features of AD pathology. Mice at early and advanced disease stages were chronically treated with an ADP receptor antagonist, and the effects on cognitive and motor functions were assessed.

Biochemical and histological analyses were conducted to examine changes in protein markers related to neurodegeneration and neuroinflammation following treatment. Chronic ADP receptor blockade led to moderate spatial memory and motor coordination improvements in APP/Tau mice. Biochemical and histological analyses revealed significant alterations in the expression of microglial, neuronal, astrocytic, synaptic and inflammatory markers associated with AD pathology, with some changes mitigated by the antagonist.

These findings suggest that ADP receptor signalling in microglia contributes to AD pathogenesis and that inhibiting this pathway can partially restore functional and molecular deficits in APP/Tau mice. Thus, targeting microglial ADP receptors could represent a potential therapeutic strategy for AD, though further research is needed to clarify the therapeutic efficacy and underlying mechanisms of ADP receptor modulation in AD.

This work is being supported by Ministerio de Ciencia e Innovación (PID2021-125785OA-I00 funded by MICIU/AEI/10.13039/501100011033 and FEDER Una manera de hacer Europa, MP), Alzheimer Association (AARG-22-968879, MP), FI Grant (2024 FI-1 00935, YJ), and UF grant PRE2022-102708 funded by MICIU/AEI/10.13039/501100011033 and by ESF+.

Targeting neuroinflammation to preserve neuronal network activity in epilepsy

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Epilepsy is the third most common chronic brain disorder and is described as a chronic brain disorder where normal neurodynamics transform into pathodynamics after a process of epileptogenesis, triggering recurrent spontaneous unprovoked electrical seizures. These seizures occur in a positive feedback loop of various factors, including neuroinflammation. Among other pathways involved in neuroinflammation, evidence shows the involvement of the JAK/STAT signaling pathway in epilepsy, although knowledge of this participation is limited.
This project is based on evidence suggesting the possible relevance of the STAT3 transcription factor in epilepsy. In this project, an in vivo model of medial temporal lobe epilepsy (MTLE) was tested where WP1066 (a STAT3 inhibitor) was administered, with the hypothesis that this inhibitor could attenuate the alterations observed in the hippocampal neurogenic niche of the MTLE murine model. To this end, MTLE was induced in mice by injecting kainate, with WP1066 treatment beginning the day before the injection, and continuing for up to 14 days. The observed alterations were analyzed by immunofluorescence.
The murine model of MTLE due to kainate mimics the alterations in the hippocampal neurogenic niche observed in MTLE patients. STAT3 blockade efficiently reduced the in vivo granular layer dispersion that occurs in MTLE; prevented the reactivity response of astrocytes and the degeneration of GABAergic neurons; and reversed the neurogenic capacity as well as the dispersion of immature neurons in the granular layer. This project provides evidence that blockade of the STAT3 signaling pathway by WP1066 may prevent some of the hippocampal neurogenic niche alterations characteristic in MTLE. The development of compounds that block STAT3 could result in therapies to modify the characteristics of epilepsy and not only treat the symptoms.

The astrocyte-to-neuron L-serine shuttle contributes to hippocampal plasticity

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Aside from ATP production, a major function of glycolysis is to provide precursors, such as L-serine, to support macromolecular synthesis. L-serine is a metabolic hub and plays pivotal role in the brain. It is the precursor of D-serine, the principal co-agonist of synaptic N-methyl D-aspartate receptors (NMDARs) required for the induction of long-term synaptic plasticity in the hippocampus. We found that the phosphoglycerate dehydrogenase (Phgdh), the first enzyme of the L-serine pathway, is mainly expressed in astrocytes in mouse and human brain and is critically involved in both long-term synaptic plasticity and spatial memory. We then explored the role of L-serine in pathological conditions leading to cognitive deficits such as Alzheimer's disease (AD). This disease is characterized by an early decrease of brain glucose metabolism in specific brain regions. We found that 3xTG mice (a genetic model of AD), at early stage of the disease, exhibit decreased L-serine levels in the hippocampus, a region where glucose metabolism in vivo is reduced, but not in the frontal cortex, which does not show any metabolic alteration. Accordingly, acute application of D-serine or L-serine rescues long-term synaptic plasticity and chronic oral L-serine supplementation restores memory deficits in AD mice. We are currently studying whether and how L-Serine and Lactate, another well-studied 3-carbon metabolite derived from the glycolytic conversion of glucose in astrocytes, cooperate to regulate synaptic activity.

The presynaptic dimension of brain pathologies: Munc13-1 and Complexin and their control over synaptic (dys)function

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A great progress to decipher the molecular content of the neurotransmitter release machinery, the SNARE-machinery, has been done. However, to date, which proteins or isoforms are essential for which synapses and how synaptic dysfunction leads to brain disorders is essentially unknown in most cases. Our studies are based on the hypothesis that differences in synapse behavior are attributed to distinct molecular composition of the neurotransmitter release machinery, resulting into a wide spectrum of reactions for synaptic vesicle preparation and fusion. Accordingly, pathological changes in the expression of protein isoforms likely distorts the pattern of neural communication by certain synapses more than others, leading to microcircuit imbalance and disease. The general aim of our studies is to gain insight into synapse-specific (dys)function and its relevance for network (patho)physiology. In particular, we investigate which synapses in the cerebellum and hippocampus are functionally vulnerable when neurotransmitter release is compromised. We focus on complexin-1-(Cplx1) and Munc13-1, key constituents of the SNARE-machinery. Recently, it was proposed to define as 'SNAREopathies' those brain disorders caused by mutations in the functionally related SNARE machinery, among which Cplx1 and Munc13-1 are susceptible proteins. Mutations in both genes lead to severe clinical defects in human individuals characterized by motor and cognitive disorders. Through mouse models of knock-out and knock-in that recapitulate the symptomatology observed in SNAREopathy patients, and by using challenging and specialized electrophysiology on hippocampal and cerebellar slices, we have found that specific synapses are mostly vulnerable when lacking Cplx1 or Munc13-1, and show a dramatic reduction in synaptic strength and a shift in short-term plasticity, which, in turn, changes the pattern of synapse communication within nerve microcircuits. Our functional studies are combined with mathematical models, fluorescence and electron microscopy, and animal behavior. Altogether, we have found that Cplx1 and Munc13-1 are essential factors for healthy brain computing.

The Role of PSA-NCAM in the Thalamic Reticular Nucleus: Implications for Inhibitory Circuits and the Impact of Early-Life Stress

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Exposure to early-life stress can profoundly impact brain development and behavior, potentially contributing to mental disorders. Both clinical and animal studies have demonstrated that early-life adversity can alter parvalbumin (PV)-expressing neurons, which play a crucial role in these disorders. During early postnatal development, PV+ neurons undergo a prolonged maturation process, particularly in areas like the thalamic reticular nucleus (TRN), a region composed mainly of GABAergic neurons, many of which express PV. This developmental stage makes these neurons especially vulnerable to early-life stress. The function of PV+ neurons is influenced by various factors, including components of the extracellular matrix and plasticity-related molecules like polysialylated neural cell adhesion molecule (PSA-NCAM). PSA-NCAM plays a significant role in neurodevelopment and the regulation of connectivity in adult inhibitory neurons, including PV+ cells. However, the expression of PSA-NCAM in the thalamus and its specific role in TRN physiology, particularly in PV+ cells, remains insufficiently understood. To address this, we explored the role of PSA-NCAM in the TRN, examining its postnatal development, potential involvement in sensory gating, and the effects of early-life stress on its expression. We analyzed PSA-NCAM expression across postnatal and adult stages, investigating its colocalization with PV+ cells, synaptic markers, and perineuronal nets (PNNs), specialized extracellular matrix structures surrounding PV+ cells in the TRN. We also enzymatically depleted PSA from NCAM specifically in the TRN and assessed its impact on TRN function. This depletion led to behavioral and histological changes, suggesting that PSA-NCAM is essential for the physiology of PV+ cells and TRN function. Notably, depletion increased the expression of FosB in PV+ cells and enlarged the area covered by NMDA receptors and PNNs. Lastly, we found that postweaning social isolation increased PSA-NCAM expression in the dorsal TRN, indicating that early-life stress influences its regulation.

The Synaptic Role of NG2-Glia Cells in the Olfactory Bulb

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NG2-glia recently emerged as one of the most multifaceted and enigmatic groups of macroglia in the CNS. Traditionally categorized as oligodendrocyte precursor cells (OPCs), recent findings suggest that NG2-glia serve a wide range of functions beyond their original characterization. These roles include acting as potential multipotent progenitors, aiding in cell migration during development, and releasing neuroprotective factors. However, their most intriguing and widely debated function involves their potential involvement in regulating synaptic activity.
NG2 glia have been observed to interact with various synaptic components, suggesting a deeper involvement in neuronal communication. Due to the inherent complexity of the synaptic network, and the heterogeneous nature of NG2-glia, key questions remain unanswered: whether individual NG2-cells can participate in both GABAergic and glutamatergic synapses, whether they act exclusively as postsynaptic components, or if their role in synaptic regulation is linked to their broader physiological functions.
In this study, we further investigate the relationship between NG2 glia and the synaptic connectome of the mouse olfactory bulb (OB), a region characterized by its unique circuitry and ongoing adult neurogenesis. These features make the OB an ideal model for examining the synaptic functions of NG2-glia. To assess the distribution of NG2 cells and synaptic markers in detail, we performed immunohistochemical staining with five distinct synaptic markers in PDGFRα-CreERT-TdTomato brain. Using advanced high-resolution imaging techniques, such as TauSTED-Xtend, we captured detailed images of NG2-cells and the surrounding synaptic markers across the OB layers. To analyze potential colocalization between NG2-glia and synaptic markers, we employed an automated, distance-based approach to quantify the number and proximity of synaptic puncta of each marker for further 3D-reconstruction. Our findings advance our understanding of their diverse contributions to neural function, concluding that NG2-glia are integral to neural communication playing a more complex role in synaptic regulation than previously recognized.

Supported by PID2022-136882NB-I00 funded by MICIU/AEI/10.13039/501100011033. R.F.A. was funded by an FPI grant from the Ministerio de Ciencia, Innovación y Universidades.

Transcranial Static Magnetic Stimulation Selectively Modulates Neuronal Activity and Boost Sensory Responses in Awake Mice

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Transcranial static magnetic stimulation (tSMS) is a promising non-invasive neuromodulation technique that applies neodymium magnets (120–200 mT) to the scalp, generating sustained magnetic fields capable of modulating neuronal activity. Despite increasing interest, its mechanisms of action and effects on neuronal firing and sensory processing remain incompletely understood. Investigating how tSMS affects cortical circuits is crucial for assessing its potential in both basic neuroscience and clinical applications.

This study examines the immediate and aftereffects of 1 hour of tSMS over the primary somatosensory cortex (S1) in awake, head-restrained mice using high-density electrophysiology system Neuropixels 1.0. Neuronal activity and sensory-evoked potentials (SEPs) elicited by whisker stimulation were analyzed in experimental (n=11) and sham (n=11) groups. Neurons were sorted with Kilosort and classified as pyramidal or narrow interneurons based on electrophysiological properties analyzed with CellExplorer.

Firing rate across all S1 neurons increased during tSMS (10.05 ± 2.15%; n=203). When analyzed by cell type and layer, the most pronounced increase was observed in pyramidal neurons of layer II/III (26.89 ± 14.33%; n=15) and in interneurons of layer V (17.50 ± 3.18%; n=29), suggesting distinct layer- and cell-type-specific responses. SEP analysis revealed a more pronounced amplitude increase in layer II/III (12.87 ± 3.47%; n=11), while the increase observed in layer V (9.03 ± 4.23%; n=11) was less prominent compared to the other layers.

These findings suggest that tSMS selectively modulates cortical excitability, with distinct effects depending on neuronal type and cortical layer. The sustained changes in pyramidal and interneuronal activity, particularly in layer II/III and V, indicate a direct influence on sensory processing. Understanding these cell-type-specific effects is crucial for unveiling the underlying mechanisms of tSMS-induced plasticity and its potential in neuromodulation strategies. Further research will help determine its applicability in both basic neuroscience and translational approaches.

TRESK potassium channel modulates hippocampal neuronal excitability and synaptic plasticity, along with sex-dependent behavioral changes

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The brain's physiological dynamics depend on ion channel activity which finely tunes neuronal intrinsic excitability, regulating the excitatory-inhibitory balance, and ultimately shaping behavior. Of particular interest is controlled potassium current leakage via K_2P channels. TRESK (K2P18.1) has a pivotal role as a brake for nociception at the peripheral level. Intriguingly, despite its widespread expression throughout the central nervous system, its function within the brain remains largely unexplored. This study investigates the functional impact of TRESK on neuronal excitability, synaptic plasticity, and behavior. We combined RNAscope in situ hybridization for expression pattern identification, electrophysiological recordings in acute hippocampal slices for neuronal excitability assessment, and a battery of behavioral tests to investigate the effects of TRESK deletion on mouse behavior. We observed TRESK mRNA throughout the hippocampus, and field-potential and patch-clamp experiments in TRESK KO mice revealed that the absence of TRESK increases neuronal excitability in CA3 pyramidal neurons and alters short-term (paired-pulse facilitation) and long-term synaptic plasticity (LTP, LTD) in the hippocampus. Behavioral analyses showed sex-dependent alterations in cognitive function and emotional regulation in TRESK KO mice. Mice lacking TRESK displayed impaired sociability in the three-chamber social test, suggesting that TRESK plays a role in social behavior. In the elevated zero maze, female KO mice exhibited increased anxiety-like behavior, spending less time in open areas. In the Y-maze test, KO mice showed enhanced spatial working memory performance, evidenced by increased spontaneous alternations. These findings highlight TRESK as a modulator of hippocampal neuronal function and suggest that disruptions in its activity may contribute to sex-dependent behavioral changes. These data provide a foundation for future studies on the role of TRESK channels in intrinsic excitability, which will provide insights into principles governing the brain's dynamics.

Uncovering Striatal Glia impact of Parkinson's Disease by sn-RNA-seq

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Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons in the Substantia Nigra, leading to basal ganglia dysfunction and impairments in the dorsal striatum, which consists of the Putamen (Pu) and Caudate Nucleus (CN). Neuroinflammation, driven by astrocytes and microglia, plays a key role in PD pathology, yet their specific contributions remain incompletely understood.

Using single-nuclei transcriptomics, we identified distinct astrocyte and microglial PD-specific subpopulations. We identified six astrocyte subtypes (A0-A5), each defined by unique transcriptional signatures and functional specializations. A0 astrocytes are involved in metabolic homeostasis and neurovascular regulation, while A1 astrocytes are associated with synaptic communication. A2 astrocytes exhibit neuroprotective and stress-responsive properties, whereas A3 astrocytes contribute to structural support and synaptic remodeling. A4 astrocytes, identified predominantly in CN (both in Control and PD), are linked to ion homeostasis, mitochondrial function, and cilia-related processes, potentially associated with lateral ventricle functions as demonstrated by spatial transcriptomics. In Pu, an increased presence of A2 astrocytes suggests a cellular response to neurodegeneration, while a diminished A3 astrocytes indicates potential synaptic dysfunction. A5 astrocytes are implicated in neurovascular integrity, blood-brain barrier function, and synaptic plasticity. Spatial transcriptomics confirmed these patterns, highlighting the activation of the unfolded protein response and the emergence of reactive astrocyte phenotypes in PD.

We also identified five distinct microglial populations, including homeostatic (M0), stress-responsive (M1), metabolically adaptive and neuroprotective (M2), phagocytic/inflammatory (M3), and synapse-associated (M4). A significant loss of M0 microglia in both Pu and CN suggested impaired neuronal maintenance, while M2 microglia exhibited transcriptomic alterations and sex-specific changes.

These findings reveal that PD disrupts astrocyte and microglial homeostasis in a region- and subtype-specific manner, contributing to neuroinflammation, metabolic stress, and synaptic dysfunction. Understanding the heterogeneity of glial responses in PD may provide novel therapeutic insights.

Unraveling UNC13A-related neurotransmission defects in a TDP-43 proteinopathy mouse model

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UNC13-A/Munc13-1 is essential for synaptic vesicle priming and neurotransmitter release, particularly at excitatory synapses. Recently, it has been linked to neurodegenerative diseases, collectively known as TDP43-proteinopathies such as, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and Perry syndrome. Furthermore, UNC13A has been implicated in diseases where TDP-43 abnormalities coexist with tau pathologies (Multiple Etiology Dementias, including Alzheimer's disease).

In these diseases, UNC13A/Munc13-1 dysfunction is associated with the loss of TDP-43 from the nucleus. Under physiological conditions, TDP-43 ensures proper RNA splicing, producing functional UNC13A RNA and its corresponding protein. However, in pathological conditions, dysfunctional TDP-43 is depleted from the nucleus and sequestered in the cytoplasm as insoluble aggregates, leading to aberrant splicing of UNC13A. This mis-splicing introduces a “cryptic exon” at intron 20-21, inducing the degradation of UNC13A RNA and a subsequent loss the protein. UNC13A disruption in these diseases may impair neurotransmitter release, particularly in synaptic vesicle priming, and could affect other neurotransmission UNC13A-related stages. However, the precise mechanism by which nuclear TDP-43 depletion alters the different stages of neurotransmission remains unclear.
Neuronal autaptic cultures, where isolated neurons establish synapses with themselves, provide an excellent model for studying neurotransmission at single cell level. Using cortical tissue from TDP-43 conditional knock-out mice, will clarify the effects of TDP-43 nuclear depletion in glutamatergic and GABAergic neurotransmission during electrophysiological recordings.
We aim to conduct electrophysiological recordings to assess whether TDP-43 dysfunction in autaptic cultures of this mouse model alters key neurotransmission parameters associated with UNC13/Munc13-1, such as synaptic vesicle priming, spontaneous neurotransmitter release, evoked calcium-dependent neurotransmitter release, and synaptic efficiency (vesicular release probability, presynaptic short-term plasticity). These recordings enable us to distinguish between excitatory and inhibitory neurotransmission, providing insights into how TDP-43 depletion affects neurotransmission at the individual neurons.

SENSORY AND MOTOR SYSTEMS

A collicular circuit for routing visual distractions into goal-dependent behavior

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Can we predict when a visual distractor will capture our attention? Visual distractors can trigger overt responses or remain unattended. The circuitry underlying this action selection remains poorly understood due to the predominant use of paradigms restricted to head-restrained animals in multisensory tasks, while relevant visual behaviors in mice are centered around fear or escape responses. Here, we demonstrate a novel paradigm using self-initiated visual search to trigger animal navigation, enabling the randomized mid-flight presentation of salient visual stimuli to assess distractibility within the context of goal-dependent behavior. Computer vision methods were applied on imaging data to capture behavioral state transitions, which ranged from attentive distractor engagement to trajectory perturbations or stimulus neglect. To quantitatively characterize these behavioral states, we derived multiple kinematic metrics from the animals' movement patterns. This analytical framework enabled implementation of linear discriminant models to classify visual distraction intensity based on motion signatures during distraction phase, and to predict distraction level from preceding search-related behavioral patterns. During this attention-distractor paradigm, we manipulated and recorded from glutamatergic (vGluT2), including medullar projection-specific cells, and GABAergic (vGAT) neurons in the motor-related layer of the lateral superior colliculus (l.SCm) to study whether this conserved circuitry is capable of tuning visual distractors in and out from behavior. In vivo cell-type-specific calcium recordings -via fiber photometry- reveal their involvement to this behavior, while their optogenetic and chemogenetic manipulation (activation and/or inhibition) enables direct control of visual distractor resilience allowing for the selective increase or decrease. We propose that local l.SCm inhibition can set a visual distractor alert-to-suppress threshold during task-dependent behavior.

All-optical Circuit Interrogation of Visual and Frontal Inputs to the Intermediate Superior Colliculus

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Since the dawn of vertebrate evolution, neural circuitry embedded within the midbrain superior colliculus (SC) has evolved to support visual-based orienting behavior and is involved in salience detection and attention-distractor processes. The lateral division of the intermediate SC (l.SCi) contains both excitatory (E: vGluT2+) and inhibitory (I: vGAT+) neurons, serving as a brain-wide integration hub that receives inputs from visual, frontocortical, and nigral regions.
In this study, we aim to determine the integration modes and selective activation patterns of visual inputs arising from the retina and higher visual areas (HVAs), as well as frontal inputs (M1 and mPFC), onto E and I neurons in the l.SCi using opto-electrophysiology and single-cell imaging (calcium/voltage) ex vivo. To better understand whether these inputs cooperate (via linear/nonlinear summation) or compete (via local inhibition), we will co-activate cell-type-specific inputs and record the response profiles of E/I neurons in the l.SCi using high-density Neuropixels recordings.
So far, our data suggests that M1-specific input bypasses local GABAergic SCi populations, whereas anterolateral HVA input engages both E and I populations in the l.SCi. This study aims to deepen our understanding of how phylogenetically older circuits and neocortical circuits coordinate, cooperate and compete, which is crucial for comprehending the computation of visual salience.

Analysis of whisker somatosensory input's role in the generation of head egocentric reference.

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The whisker system is used by rodents to explore the environment, sensing the presence of objects along their path. Sensory information collected through vibrissa is relied to the contralateral barrel cortex (BC) following a somatotopical organization; being a highly lateralized system. However, functional and anatomical differences have been described between the subregion of the BC associated to whiskers in distinct rows. Previous work in our group has shown that the barrels processing the input of medially located follicles have a more relevant role in callosal-mediated interhemispheric communication than lateral ones (Montanari et al., Sci. Adv. 2023). Therefore, they are a candidate to be involved in actions that require bilateral integration between hemispheres. In humans, alterations in the corpus callosum connecting visual areas influence depth perception, demonstrating that callosal inputs have a functional role. Based on our previous results, we hypothesize that in the case of the whisker system, highly interhemispherically connected areas could be associated to the generation of an egocentric reference of the midline of the face in the context of object localization. To interrogate this hypothesis, we have designed a behavioral paradigm in which freely moving mice approach a waterspout they have been trained to locate, measuring the time spent in successfully reaching it under different perturbations of the whisker system. Moreover, by using a high-speed infrared vision camera, we track movement patterns that reflect the impact of the missing whiskers input. Our preliminary results show an impairment on the task performance when blocking medial whiskers sensory transmission, in addition to higher exploration of landmarks in the arena with the untouched whiskers to compensate for the missing afference. All in all, we are characterizing the differential contribution of the distinct rows of the whisker system to the generation of a head egocentric spatial reference in mice.

Auditory Stimulus-Specific Adaptation in the primary visual cortex of the Anesthetized Rat

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As humans and other living organisms interact with their environment, the brain tries to generate an elaborate model of the world in the most efficient manner, by integrating sensory information and adapting to change. Primary sensory cortices are known to respond to cross-modal stimuli, such as auditory input in the auditory cortex (AC) and visual cortex (V1), but the broader networks supporting multisensory cognition remain elusive.
The brain automatically detects auditory changes, as reflected in mismatch negativity (MMN), a key biomarker of deviance detection. At the neuronal level, stimulus-specific adaptation (SSA) is considered MMN's cellular counterpart. Studies of cross-modal processing in animal models indicate that multisensory interactions influence MMN with minimal top-down modulation from attention and prior knowledge.
To investigate V1 neurons' role in auditory deviance detection, we first recorded their frequency response areas to assess auditory sensitivity. We then applied the auditory oddball paradigm with control sequences (many-standards and cascade) in anesthetized rats. Pure tones (75 ms) were presented at 1 Hz while we recorded neuronal spiking activity in the V1 using 32-channel probes and tungsten electrodes. We observed distinct SSA responses, long-lasting auditory responses (~175 ms latency, peaking at ~400 ms), and significant SSA indices in a subset of auditory-responsive neurons. V1 neurons distinguished between random and periodic oddball responses, suggesting sensitivity to auditory pattern regularity.
The presence of auditory SSA in V1 supports its role in a distributed deviance detection network across sensory modalities, emphasizing the complex neural circuits involved in multisensory processing and mismatch negativity.

Financial support was provided by the Spanish Ministry of Science and Innovation (MICIN; grant # PID2019-104570RB-I00); and Consejería de Educación, Junta de Castilla y León (grant # SA218P23); JSS held a fellowship PRE2020-095617 funded by MCIN/AEI/ 10.13039/501100011033 and by "European Union NextGenerationEU/PRTR".

Cellular and Molecular Mechanisms Involved in Widespread Cortical Remodeling in the Adult Brain

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Brain plasticity, or the ability of the brain to remodel itself and adapt to life experiences, is most pronounced during development. As the brain matures, neural networks become highly refined and specialized, leading to a reduction in structural dynamism, largely perceived as a static network of cells. Previous data from the team shows that peripheral injury to sensory organs in the adulthood triggers wide remodeling of cortical circuits, affecting neuronal activity and connectivity in primary and secondary sensory areas. Because the adult brain is a highly specialized organ, with a complex extracellular matrix and limited expression of guidance systems, how long-range reorganization in these mature circuits can be implemented, is an open question. To investigate the molecular and cellular fingerprint of adult cortical remodeling, we used a previously established model of permanent whisker deprivation in adult mice, and combined it with cutting-edge whole-brain in situ hybridization and immunostaining techniques (TRIC-DISCO and iDISCO+, respectively), classic 2D histology and advanced bioinformatics tools for large-scale image segmentation (ClearMap toolbox). Our results reveal the multifactorial nature of adult circuit remodeling, involving changes in the distribution of astrocytes and microglia, while notably, no topological alterations are observed in the vascular network. Although subtle and slow, changes in adult networks may have major functional implications, and could hold the key to inspiring new therapeutic approaches targeting the adult brain.

Combinatorial Biomaterial and Motor Training Therapy Promotes Supraspinal Plasticity and Functional Recovery After Spinal Cord Injury

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Piezo4Spine aims to develop a multifactorial therapy to promote axonal regeneration and brain-spinal cord connectivity after spinal cord injury (SCI). Our first step was the development of a 3D-biomimetic collagen-based matrix with iron oxide nanoparticles (COL-CHI), combined with a motor training protocol. After in vitro characterization of the matrix components showing no impairments on neuronal activity or connectivity, the biomaterial was implanted in vivo in a cervical hemisection model (C6) across different groups: SCI, SCI + COL-CHI and SCI + COL-CHI + motor training (combined therapy). These animals underwent longitudinal assessment up to 120 days post-injury to assess sensorimotor functions and thalamocortical connectivity using electrophysiology, behavior and anatomical approaches. Tactile and thermal behavioral tests showed altered sensory threshold responses in COL-CHI rats, particularly in the dorsal column-lemniscal (tactile) pathway, while the spinothalamic (temperature) pathway was less affected. Additionally, COL-CHI rats showed improved forelimb motor abilities in a task assessing corticospinal tract function. Both sensory and motor improvements were observed up to 60 days post-injury but decreased at late stages. This chronic effect was probably due to the progressive COL-CHI scaffold degradation, as evidenced by histological analysis of the spinal cord showing the presence of cavities within the hydrogel. However, the chronic changes at behavioral level were reverted in animals submitted to motor training protocols, suggesting that the combinatorial therapy counteracts biomaterial degradation effects. At circuit level, COL-CHI and COL-CHI + MT rats showed increased sensorimotor plasticity evidenced by enhanced magnitude of cortical sensory evoked potentials in in vivo electrophysiology and increased thalamic inhibition by immunofluorescence staining of GABAergic afferents in NRT, POm and VPL. Together, these results demonstrate that biomaterial degradation should occur only after the full recovery or stabilization of spinal cord circuits and that combinatorial therapy promotes supraspinal plasticity that persists even after biomaterial degradation.

Correlation of Alzheimer Disease and hearing loss. Role of Kv3 channels in the auditory pathway of Alzheimer mice models

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Deafness is one of the most common illnesses in old age, with presbycusis or age-related hearing loss being the most frequent cause. Various studies have shown that hearing loss significantly influences the isolation situation of the elderly. In the late eighties, it was revealed that the association between neurodegenerative diseases and deafness seemed to predispose individuals to a more accelerated cognitive decline. In the auditory system,the intrinsic electrical properties and synaptic integration in neurons are mainly governed by a wide array of voltage-dependent ion channels. studying this channels can determine if the alteration in signal transmission of these neurons can be a marker for the early detection of neurodegenerative diseases such as Alzheimer Disease (AD).

For this purpose, we studied the expression of KCNC1 (Kv3.1) and KCNC3 (Kv3.3) channels, channels necessary for the correct hearing transmission, in the auditory cortex and inferior colliculus of 6-month old and 18-month old Alzheimer mice models and compared with the control group. We also determined the expression of these channels in different glial cells and in vitro, we determine the relation of this channel with the increase of amyloid precursor protein (APP) expression and amyloid beta formation.

We found an increase Kv 3.1 and Kv3.3 channels expression mostly in 18-month-old AD mice in the auditory cortex and inferior colliculus compared to the control group. Moreover, this overexpression was only in neurons but not in astrocytes or glia. Indeed, in vitro analysis revealed a correlation between APP and this channel.

In conclusion, the present work supports the hypothesis that there is a correlation between ion channels expressed in the auditory pathway, necessary for the correct hearing transmission, and AD. Moreover, there is a link between the APP and Kv channel function, revealing that they may have a role in the hearing loss after AD.

Cutaneous Schwann Cells sensitivity to oxaliplatin

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Traditionally, nerve-associated Schwann Cells (SC) have been thought to either myelinate surrounding nerve fibres, gather small nerves, repair injured nerves, or maintain nerve terminals at different body regions. Besides these canonical roles, a major role of SC in nociceptive stimulus perception has been described. Since then, several other papers have come out trying to solve questions related to how this SC subpopulation, known as nociceptive SC (nSC), takes part in detecting, transmitting or modulating nociceptive stimulus. In fact, it has already been discovered the nSC involvement in some peripheral neuropathies, such as Diabetes Mellitus Type I-induced. Thus, researchers have come up with different hypotheses stating that nSC could be crucial in understanding how peripheral neuropathies develop or become chronic, and also how they could be targeted. Among the most frequent neuropathies, chemotherapy-induced peripheral neuropathy (CIPN) is one of the most concerning, as it may hinder adequate cancer treatment. Epidemiological data show that up to 90% of patients treated with oxaliplatin and paclitaxel develop CIPN. Although it is well documented that chemotherapeutic agents induce changes in protein expression in nociceptive nerve fibers, consequently affecting nerve excitability and sensitivity, the reported changes primarily involve thermal hypersensitivity. However, the cellular and molecular mechanisms underlying CIPN-induced mechanical hypersensitivity remain unclear, potentially reflecting a more complex scenario in which nSC may play a central role. We are investigating how CIPN alters nSC physiology, modifying their sensitivity to different sensory stimuli and affecting the functional glial-neuronal complex established between nSCs and subcutaneous free nerve terminals. To address this question, we use the oxaliplatin CIPN mouse model with the SC green fluorescent reporter line Plp-eGFP. Also, we have validated cutaneous SC sensitivity to oxaliplatin in primary culture and preliminary electrophysiological data indicate that oxaliplatin increases mechanical sensitivity in SCs. The exact molecular basis is currently under investigation.

Deciphering conflicting data regarding ageing and pain sensitivity

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Chronic pain has been associated with ageing and cognitive impairment, as illustrated by the prevalence of chronic pain in Alzheimer's disease (AD), which is around 45.8%. In concomitance, the nociceptive system is strongly intercommunicated with the immune system through proinflammatory cytokines, capable of sensitizing non-nociceptive peptidergic cells and eliciting the production of more cytokines. This loop provides a basis for the early development of cognitive impairment in a chronic inflammatory setting. Our hypothesis is the possible existence of differences in the expression of nociceptive peptides (or their receptors) that are accentuated with aging and related to chages in the nociceptive threshold. To verify our hypothesis, we tested mice from an AD mouse model (APP/PS1) and compared them with C57BL/6 controls for their pain sensitivity (Von Frey test) and expression of nociceptive markers in the dorsal root ganglion cells (NK1R, RAMP1, CGRP, P-substance and NPY) by using immunohistochemistry. In addition, we assessed the expression of nociceptive markers in the dorsal root ganglia and the posterior horn of two groups of human cadavers (60-70 years old and 80-90 years old). First, Von Frey test did not show age-related differences in the nociceptive threshold for mechanical stimulation between C57BL/6 and APP/PS1 mice. Second, in both C57BL/6 and APP/PS1 mice, a trend toward an increase in the number of DRG cells expressing NK1R was observed, whereas in human DRG, both RAMP1+ and NK1R+ appeared to be increased and correlated with age (ρ=0.529, sig.=0.008 and ρ=0.427, sig.<0.038). Our results show that, although ageing is related to an increase of mostly CGRP-related markers in sensory structures, this increase is unrelated to increased pain sensitivity

Distribution of the noradrenaline innervation in the mouse thalamus

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Noradrenaline is a modulatory neurotransmitter that plays a crucial role in a wide variety of brain functions, including sensory processing, arousal, sleep-wake cycles, stress and anxiety regulation, and memory formation. Many of these functions involve circuits that include the thalamus. However, a detailed description of the distribution of noradrenergic axons in the mouse thalamus—one of the most widely used animal models for studying brain physiology and pathology—remains lacking.

In this study, we mapped and characterized the distribution of noradrenergic axons in the mouse thalamus. To achieve this, we performed immunohistochemistry against the noradrenaline transporter (NET). Maps of the distributions of noradrenaline axons in the thalamus were built from micrographs of the processed tissue using Neurolucida and Ilastik software.

Noradrenaline axons were present in almost all thalamic nuclei at moderate to high densities. The highest densities were observed in the anterior nuclei, midline nuclei, some intralaminar nuclei (central lateral, paracentral, parafascicular), and the dorsal lateral geniculate nucleus. In contrast, certain regions of the posterior nucleus exhibited the sparsest noradrenergic innervation. Some of these patterns are similar to those present in the primate thalamus (midline, intralaminar), while others differ significantly (e.g., dorsal lateral geniculate and anterior nuclei).

Our findings suggest that noradrenaline plays a key role in modulating limbic circuits through the thalamus, including those related to memory acquisition. However, species-specific differences in noradrenaline distribution across certain nuclei highlight the need for caution when extrapolating findings from mouse models to the human thalamus.

Supported by the Chair in Neuroscience UAM-Fundación Tatiana.

Elucidating an anterior cingulate circuit for self-initiated actions and rescue of Parkinsonian akinesia

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Dopamine (DA) depletion is known to result in Parkinsonian symptoms such as the inability to initiate movements (akinesia). Such symptoms are usually associated with reduced DA signaling in the striatum. The frontal cortex, which projects strongly to the striatum, has also been implicated in initiating volitional actions, but its precise role remains poorly defined. Here we show that a specific cell population expressing D1-like dopamine receptors in the anterior cingulate cortex (ACC) plays a critical role in movement initiation. Activating these neurons flexibly generates targeted movement, and is sufficient to rescue complete akinesia induced by DA receptor antagonism or DA depletion. Our results reveal a new circuit mechanism for movement initiation and suggest novel treatment strategies for Parkinson's disease.

Evaluation of the therapeutic potential of hMSC-derived secretome for RPE regeneration in AMD

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The retinal pigment epithelium (RPE) is a monolayer of polarized cells essential for retinal homeostasis, primarily through interactions with photoreceptor outer segments. RPE dysfunction plays a key role in the onset of retinal pathologies such as age-related macular degeneration (AMD). Among potential therapeutic strategies, human bone marrow-derived mesenchymal stromal cells (hMSC) have demonstrated beneficial effects when administered subconjunctivally, largely attributed to their secretome (trophic factors and extracellular vesicles (EVs)).

This study aims to evaluate the impact of hMSC-derived secretome on autophagy regulation in an in vitro model of pathological RPE associated with AMD. A human induced-pluripotent Stem Cells (iPSC)-derived RPE model, maintained in transwells, was established and characterized, exhibiting molecular and physiological features of a polarized human RPE monolayer. To mimic early AMD conditions, the model was exposed to human sera from both healthy individuals and patients with dry or exudative AMD.

In this pathological RPE model, we analyzed the therapeutic potential of hMSC-derived secretome by direct administration to in vitro RPE-AMD models. Autophagic activity was evaluated using fluorescence microscopy with specific autophagy markers and quantified by western blot. Additionally, intra- and intercellular changes induced by secretome administration were analyzed by using fluorescence microscopy. Furthermore, the composition of the secretome and EV profiling were characterized using proteomics and the Exoview System, a novel technology enabling high-resolution EV detection for accurate and complete characterization of the entire profile of both membrane and intracellular proteins.

This established model allows the identification of key factors involved in RPE recovery, mediated by the regulatory effects of hMSC. Furthermore, it provides a valuable platform for testing potential treatments for AMD and other retinal degenerative diseases. By facilitating controlled studies of cellular responses to therapeutic agents, this model can contribute to the development of novel regenerative strategies, ultimately advancing treatment options for patients with retinal pathologies.

External activation of the auditory cortex through anodal DC stimulation stabilizes cortical effects of aging after bulk RNA – seq analysis

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In a model of natural aging in Wistar rats, we previously demonstrated that multisession direct current stimulation (ES) of the auditory cortex (AC) preserves hearing thresholds (Fernández del Campo, 2024) and stabilizes cortical hyperexcitability by reducing the loss of inhibitory microcircuits (Fernández del Campo, 2025).
Our hypothesis is that ES of the AC compensates for age-related maladaptive effects induced by the loss of ascending input regulation. As a result of our stimulation protocol, we predict that genomic alterations associated with aging may be reversed, leading to the stabilization of dysregulated genes involved in various functional impairments of aging, such as inflammation, mitochondrial damage, and synaptic plasticity regulation.
To test this hypothesis, we performed an RNA-seq analysis of the AC from young (YG), aged non-stimulated (NES), and aged electrically stimulated (ES) rats. After AC stereotaxic extraction, Genes with p-values ≤ 0.005 and fold changes (FC) ≥ 0.5 were analyzed.
Compared with YG, NES animals exhibited a larger number of upregulated genes, with relatively few downregulated genes. In contrast, when comparing ES to NES, most genes were downregulated (1,351 down vs. 297 up). Moreover, a subset of genes was common when comparing NES with YG and ES. All genes upregulated in the YG-NES comparison were downregulated in NES-ES and vice versa, suggesting a stabilizing effect on metabolism, ROS accumulation, hyperexcitability, and inflammation.
When comparing YG to ES, only 67 upregulated and 81 downregulated genes were detected, suggesting minimal changes associated with aging and no significant side effects following electrical stimulation.

Graphene based neurotechnology for peripheral nerve recording and stimulation

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Nanoporous graphene-based neurotechnology for intraneural peripheral nerve recording and stimulation

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Abstract
Millions of people worldwide suffer from loss of motor function due to nerve injuries, which drives the need for new rehabilitation therapies. Neuroprosthetic devices that interface with the peripheral nervous system (PNS) aim to restore movement by stimulating the patient's nerves to control limb motion, while other electrodes provide access to sensory feedback from the limb, creating a bidirectional interface with the nervous system. Decades of research have enabled the development of a variety of devices to interface peripheral nerves; however, their clinical use is limited due to several technological challenges. These include difficulties in recording nerve signals with high fidelity, stimulating small groups of nerve fibers with sufficient selectivity while minimizing side effects, and ensuring the long-term stability of flexible implants.
In this work, we propose the use of microelectrode thin film technology based on reduced graphene oxide, a highly porous material that offers exceptional charge injection limit (CIL) and very low impedance, enabling bidirectional high-resolution recording and stimulation. Our thin film technology consists of 10 µm thin, highly flexible neural leads featuring a high-density array of microelectrodes of 80 µm in diameter. The implementation of inorganic encapsulation layers allows long-term stability in accelerating aging tests. Impedance and CIL were monitored in both in vitro and in vivo, demonstrating long-term stability.

[1] B. Rodríguez-Meana et al., "Engineered Graphene Material Improves the Performance of Intraneural Peripheral Nerve Electrodes," Adv. Sci., vol. 2308689, pp. 1–19, 2024, doi: 10.1002/advs.202308689.

How Do We Define Dynamic Range of Complex Neuronal Response Functions?

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The neuronal response function maps external stimuli to neural activity, with dynamic range quantifying the input levels that produce distinguishable responses. Traditional sigmoidal response functions exhibit minimal firing rate changes at low and high inputs, with marked shifts at intermediate levels, making the 10%-90% response range a reliable measure. However, complex response functions, such as double-sigmoid or multi-sigmoidal profiles with plateaus, challenge conventional calculations and often lead to overestimated dynamic ranges. To address this, we propose a classification of response function complexity and introduce alternative dynamic range definitions that improve accuracy. We analyzed empirical and computational studies featuring simple and complex response functions, alongside a mouse retinal ganglion cell model that generated both response types using energy-modulating dynamical elements. We developed four alternative dynamic range definitions, which exclude plateaus to focus on discernible response variations, and evaluated their performance against the conventional definition. Our findings reveal that while the 10%-90% range is effective for simple response functions, it inflates estimates for complex profiles by including plateaus. The proposed alternatives successfully isolate meaningful response regions, maintaining accuracy for simple cases while generalizing precisely to complex ones. The neuronal model further confirmed that dendritic dynamics modifications induce complex profiles, emphasizing the need for improved measurement approaches. By refining dynamic range estimation, our study enhances the quantification of neuronal response diversity and reduces arbitrary assumptions. These methods extend beyond neuroscience, applying to fields with complex, nonlinear dynamics, with freely available computational tools facilitating adoption and refinement.

Identifying cell-type-specific alterations underlying schizophrenia-related EEG deficits using a multiscale model of auditory thalamocortical circuits

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Schizophrenia is associated with cognitive deficits, including disruptions in sensory processing. Electroencephalography (EEG) studies have revealed abnormalities in event-related potentials and cortical oscillations, particularly in the auditory system. Among the most well-established EEG biomarkers are the reduced 40 Hz Auditory Steady-State Response (ASSR) and impaired mismatch negativity (MMN). We extended our computational model of auditory thalamocortical circuits to investigate the circuit-level mechanisms underlying these deficits, refining its ability to replicate schizophrenia-related EEG features. This model simulates a cortical column with over 12k neurons and 30M synapses, incorporating experimentally derived neuron densities, laminar organization, morphology, biophysics, and connectivity across multiple scales. Auditory inputs to the thalamus were modeled using a phenomenological cochlear representation, allowing the reproduction of realistic physiological responses.

To further improve the accuracy of simulated EEG signals, we are implementing a more systematic approach to provide background network activity, representing inputs from other brain regions not included in the circuit model. We introduce time-varying, statistically independent somatic conductance injections modeled by Ornstein-Uhlenbeck (OU) processes, which mimic aggregated synaptic input while maintaining biologically realistic fluctuations. This approach improves upon previous methods by ensuring smoother, correlated variations in background activity. The mean and standard deviation of the OU process are expressed as percentages of input conductance for each cell type, linking them to intrinsic cellular properties. Additionally, we are developing an algorithm to dynamically calibrate population-specific OU parameters, ensuring flexibility as the model evolves. By integrating experimentally observed molecular and genetic alterations in schizophrenia with circuit-level dysfunctions, this work enhances the physiological fidelity of our EEG simulations. These refinements improve our ability to link cellular abnormalities to robust EEG biomarkers, providing deeper insights into the neural basis of auditory processing deficits in schizophrenia.

Neural Mechanisms Underlying Visually-Evoked Aggression in Male and Female Siamese Fighting Fish

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Aggression is a fundamental aspect of social behavior across species, influencing the dynamics of human and animal societies. While visual cues prominently trigger aggression in humans and primates, rodent and fly models, commonly used in behavioral studies, primarily rely on olfactory cues. Consequently, the impact of visual stimuli on aggression, and social behavior more broadly, remains relatively underexplored. Siamese fighting fish (Betta splendens), selectively bred for robust aggressive behavior driven by visual cues, offers a promising new model species to investigate the neurobiological underpinnings of visually-evoked aggression. This study aimed to (i) develop behavioral assays that elicit aggressive display in adult male and female and (ii) identify the neural circuits underlying visually-evoked aggression. Leveraging recent advances in machine learning, we employed markerless tracking methods to capture behavioral hallmarks of aggression (i.e. flaring). Additionally, tracing, phosphorylation of ribosomal subunit-S6 labeling, and RNA-sequencing (RNAseq) were utilized to map the neural representation of aggressive behavior. Our findings revealed that males exhibit significantly higher levels of aggression than females when visually exposed to a male conspecific. PS6 immunolabelling revealed an increase of activated neurons in the dorsomedial-pallium (Dm), and other telencephalic regions during aggressive display to a conspecific, compared to exposure to an empty tank. Furthermore, RNAseq analysis identified several differentially expressed genes (DEGs) in these brain regions between males and females, potentially contributing to sex differences in aggressive behavior. Finally, we identified distinct projections of retinal ganglion cells to lateral thalamic nucleus and optic tectum, but not to Dm, suggesting a complex neural pathway involved in processing visual stimuli triggering aggression. Our study provides valuable insights into the neurobiological mechanisms underlying sex differences in visually-evoked aggression in Betta. Future directions involve genetic manipulation of DEGs between males and females and elucidating the intersection of identified brain regions with the visual system.

Origin of the noradrenaline innervation of the midline and mediodorsal thalamic nuclei in non-human primates

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Noradrenaline (NA) modulates brain circuits, some of them through the thalamus. In primates, the NA innervation of the thalamus is heterogeneous. The midline nuclei (ML) display the densest noradrenaline innervation. Other nuclei like the mediodorsal nucleus (MD) also present significant NA innervation.

This study investigates the origin of the NA innervation to ML and MD, considering their distinct circuit connectivity and their relevance to limbic and executive functions. We used retrograde tracer injections (CtB-Au) in ML and MD, and immunohistochemistry against the NA-synthesizing enzyme DβH to identify NA neurons.

NA neurons projecting to the ML were strikingly abundant, comprising 20% of the total population of NA neurons in the brain stem. 82% of the NA neurons projecting to the ML were located in the locus coeruleus complex (LC: A6, subcoeruleus, and A4); 22% of the neurons in the coeruleus complex project to the ML. However, the contribution of other NA nuclei shouldn't be dismissed: 23% of the neurons in A7, 20% in A5, 11% in A1, and 9% in A2 projected to the ML.

NA neurons projecting to MD were also abundant, although not nearly as much as those projecting to ML. 6% of the total NA neurons in the brain stem project to MD. 90% of the NA neurons projecting to MD were located in LC, and 8% of the neurons in LC project to MD. The contribution of other NA nuclei to the innervation of MD is as follows: 2.6% of the neurons in A7, 3.6% in A5, 1.5% in A2, and 1.3% in A1 group project to MD.

Our results indicate a broader potential for NA modulation in ML compared to MD. This suggests that limbic and arousal circuits involving ML are markedly influenced by NA innervation.

Supported by the Chair in Neuroscience UAM-Fundación Tatiana.

Pedunculopontine glutamatergic Chx10+ neurons control global motor arrest in mice

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Behavioral arrest can happen upon termination of goal directed movements or as a global motor arrest either in the context of fear or in response to salient environmental cues. The neuronal circuits that bridge with the executive motor circuits to implement a global motor arrest are poorly understood. Here, we describe a global motor arrest behavior mediated by glutamatergic neurons in the pedunculopontine nucleus (PPN). We identified a population of Chx10 neurons in the mouse PPN that arrest motor behavior using immunohistochemistry and mouse genetics. We characterized the motor and autonomic features of the arrest by analyzing limb kinematics, hindlimb EMG recordings, respiratory frequency, and heart rate.
The activation of glutamatergic Chx10-derived neurons in the PPN arrests all ongoing movements while simultaneously causing apnea and bradycardia. Chx10-PPN bilateral ablation decreased natural arrest events during open field exploration. The global motor arrest has a “pause-and-play” pattern with an instantaneous halt of movement followed by a short-latency continuation from it was paused. Mice naturally perform arrest bouts with the same combination of motor and autonomic features. Chx10-PPN evoked arrest is different to ventrolateral periaqueductal gray induced freezing. Our study defines a motor command that induces a global motor arrest which may be recruited in response to salient environmental cues to allow for a preparatory or arousal state, and identifies a locomotor opposing role for rostrally biased PPN glutamatergic neurons.

Support: Novo Nordisk Foundation Laureate grant & Lundbeck Foundation R310201921

Proteomic profile of the epileptogenic nucleus in the GASH/Sal Model of Epilepsy after audiogenic kindling

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Introduction. Epilepsy is a multifaceted neurological disorder characterized by recurrent seizures and associated with molecular and immune alterations. The GASH/Sal (Genetic Audiogenic Seizure Hamster, Salamanca) provides a powerful tool to study seizure mechanisms and resistance in predisposed individuals. Objective. This study investigates the proteomic and immune responses triggered by audiogenic kindling in the inferior colliculus comparing non-responder animals exhibiting reduced seizure severity following repeated stimulation versus naïve GASH/Sal. Methods. We used auditory brainstem responses, cytokine profiling and proteomic analysis to identify key inflammatory markers and differentially expressed proteins linked to seizure susceptibility. Results. To assess auditory pathway functionality, Auditory Brainstem Responses were recorded, revealing reduced neuronal activity in the auditory nerve of non-responders, while central auditory processing remained unaffected. Cytokine profiling demonstrated increased levels of pro-inflammatory markers, including including IL-1 alpha, IL-10, and TGF-beta, alongside decreased IGF-1 levels., highlighting systemic inflammation and its interplay with neuroprotection. Building on these findings, a proteomic analysis identified 159 differentially expressed proteins. Gene Set Enrichment Analysis and Weighted Gene Co-expression Network Analysis revealed disrupted pathways related to metabolic and inflammatory epileptic processes and a module potentially linked to the rise in threshold of seizures, respectively. Differentially expressed genes, identified through bioinformatic and statistical analyses, were validated by RT-qPCR. This confirmed the upregulation of six genes (Gpc1, Sdc3, Vgf, Cpne5, Agap2, Dpp8) and the downregulation of two (Ralb, S100b), aligning with reduced seizure severity. Conclusion. This study may uncover key proteomic and immune mechanisms underlying seizure susceptibility, providing possible novel therapeutic targets for refractory epilepsy.

Pthlh Interneurons: key modulators of cognitive striatal processes

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Striatal interneurons, although low in number, are crucial modulators of MSN activity and integrate input from various brain regions to regulate basal ganglia output and behavior.

Using single-cell RNA sequencing approach we have recently described a prominent population of GABAergic interneurons expressing Pthlh in the human caudate and putamen (Garma et al., 2023) and in the mouse striatum (Muñoz-Manchado et al., 2018). These interneurons display a broad range of intrinsic electrophysiological properties that correlate with variable Pvalb expression and regional localization within the mouse striatum (Muñoz-Manchado et al., 2018).

To study Pthlh interneurons in vivo, we generated the Pthlhcre::R26R-tdTomato mouse line. We carried out high-sensitive FISH and electrophysiology recordings confirming robust labeling, around 90% of Pthlh cells are labelled with a positive correlation between reporter and Pthlh expression level, and distinct physiological properties in the dorsal striatum with specific distribution in the dorso-ventral and lateral axis, but no differences were found in the anterior-posterior. Chemogenetic inhibition (DREADDs) of Pthlh-expressing neurons using this mouse line revealed their involvement in cognitive processes such as learning and memory, as well as exploratory behavior. We also applied retrograde tracing, identifying projections from the thalamus, cortex, and amygdala, suggesting a role in integrating emotional and cognitive signals. To further map local and long-range connectivity of Pthlh interneurons, we have applied rabies virus tracing using conventional helper virus and a modified TVA helper virus to decipher the local circuit.

We have confirmed that our new mouse line is a valid tool that enables the direct study of Pthlh interneurons for the first time. This genetic tool allows us to manipulate Pthlh interneurons and thus to better understand their role in regulating basal ganglia circuitry and their potential involvement in neurodegenerative diseases in the next steps.

Sex differences in auditory brainstem responses of two rat models of autism: Environmental and genetic contribution to autism-like auditory function

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Autism is an early-onset neurodevelopmental disorder characterized by restricted, repetitive behaviors and atypical patterns of social communication and interaction. A considerable portion of autistic individuals experience divergent auditory perception, which can interfere with their ability to navigate everyday sound environments.

Auditory brainstem responses are electrophysiological potentials elicited by auditory stimuli that evaluate neural activity along the auditory nerve and brainstem. Importantly, auditory brainstem response measures vary by sex, with females typically showing higher amplitudes and shorter latencies than males. This sex-specific neurophysiological profile is especially relevant in autism research, where the male-to-female diagnosis ratio is approximately 3:1. Thus, exploring the neurobiological mechanisms underlying sex-specific variations in autistic traits is essential. Furthermore, autism sensory profiles may vary based on the independent and mutual effects of environmental and genetic factors.

To deepen this understanding, we examined auditory brainstem responses in two rat models of autism: the GRIN2B rare mutation model and the prenatal valproic acid induction model, alongside control animals. We assessed wave-specific amplitudes and peak latencies (waves I to V), inter-peak intervals (I-III, I-V, III-V), and amplitude ratios (III:I, V:I, V:III). Our findings reveal key insights into the effects of sex and genotype, as well as their interactions. Female rats generally exhibited greater amplitudes and longer latencies across all waveforms. Regarding genotype, control animals consistently showed larger amplitudes and shorter latencies compared to both autism models. Additionally, sex-by-genotype interactions played a significant role in shaping autism-related auditory phenotypes.

Acknowledgements:
We thank Drs. David N. Furness for their critical comments and constructive criticism on a previous version.

Funding:
This study was supported by projects PID2023-148541OB-I00 funded by MCIN/AEI; and Consejería de Educación, Junta de Castilla y León (SA218P23) to MSM. SCS held a fellowship from the Spanish Ministry of Science and Universities FPU2021/00124.

Stimulus-Specific Adaptation in the Hippocampus of the Anesthetized Rat

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The hippocampus is well known for its role in spatial and episodic memory, but its involvement in broader functions, including predictive processing and novelty detection, has gained recent attention. While the neural mechanisms of sound analysis are well characterized along the auditory pathway to the auditory cortex (AC) and prefrontal cortex (PFC), the broader networks supporting auditory cognition remain elusive.
The human brain automatically detects auditory changes. Predictive coding theory proposes that perception involves continuously generating and updating predictions to minimize discrepancies between expected and actual sensory inputs, with mismatch negativity (MMN) and the P300 component serving as key neural correlates of these processes. At the neuronal level, stimulus-specific adaptation (SSA) is considered MMN's cellular counterpart. Given the hippocampus's established connections with the AC and PFC, it may contribute to the generation of MMN and P300 components.
To investigate this, we applied the auditory oddball paradigm and two control sequences (many-standards and cascade) in anesthetized rats. We presented pure tones (75 ms) at 1 Hz while recording neuronal spiking activity in hippocampal CA1 and dentate gyrus (DG) using 32-channel probes and tungsten electrodes. We found that 21% of recorded hippocampal neurons exhibited significant auditory responses, with 24.3% demonstrating SSA, a neural correlate of MMN. Notably, DG neurons exhibited earlier peak activity compared to CA1, suggesting distinct temporal processing mechanisms.
These findings suggest that the hippocampus plays a role in deviance detection and predictive auditory processing, extending its known functions to include sensory prediction and adaptation.

Financial support was provided by the Spanish Ministry of Science and Innovation (MICIN; grant # PID2019-104570RB-I00); and Consejería de Educación, Junta de Castilla y León (grant # SA218P23); JSS held a fellowship PRE2020-095617 funded by MCIN/AEI/ 10.13039/501100011033 and by "European Union NextGenerationEU/PRTR".

Studying human TDP-43 in ALS and other TDP-43 proteinopathies in genomically humanised TDP-43 mouse models

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Increasing evidence suggests that alterations in TDP-43 homeostasis play a central role in the pathogenesis of ALS and other TDP-43 proteinopathies.
To try to generate the closest models to the human condition, we have successfully replaced the mouse endogenous TDP-43 gene (Tardbp) by its human counterpart (TARDBP), from the start codon in exon 2 to the stop codon in exon 6, including all introns and exons in between, within the correct endogenous Tardbp locus on mouse chromosome 4 and under the control of the mouse endogenous promoter, maintaining mouse 3'UTR. These are the first mice to express only human TDP-43 without the expression of the endogenous mouse protein. In addition, we have generated a KI mutant line carrying the M337V human mutation via genome editing with CRISPR/Cas9, of which we present its ongoing characterization to up to one year of age together with the characterization of the wild-type TDP-43 humanised mice up to 2 years of age. Further analysis will provide insights into the impact of TDP-43 humanisation and the effects of the M337V mutation on disease-related phenotypes.

Superior Colliculus Synaptic Integrity, Circuitry, and Associated-Sensorimotor Behaviour are Disrupted in Huntington's Disease

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Huntington's disease (HD) is a progressive neurodegenerative disorder characterised by motor and cognitive deficits, with early impairments in oculomotor function. The superior colliculus (SC) is crucial in integrating visual perception and motor control. Our recent observations revealed disrupted connectivity between the SC and secondary motor cortex (M2) in the HD mouse model R6/1.

We aimed to thoroughly assess SC from molecular alterations to circuit-level and sensorimotor integration disruptions. For this, we conducted histological assessment of mutant huntingtin (mHTT) aggregates, synaptic protein expression analyses in SC by western-blot, neuronal activity responses in the SC using multielectrode array (MEA) in slices, in vivo calcium recordings with fibre photometry during the Beetle-Mania Task (BMT), and whole brain SC connectivity by fMRI.

Symptomatic R6/1 mice (17-w) show mHTT aggregates in all SC layers. Presynaptic protein levels (VGLUT-1, -2, VGAT) were reduced, whereas postsynaptic PSD95 was increased. MEA recordings in SC showed modest presynaptic alterations, while in vivo fibre photometry recordings in SC showed a clear increase in calcium fluorescence in response to the beetle, although responses were similar between R6/1 and WT mice. Furthermore, R6/1 mice show reduced functional connectivity of the SC with numerous brain regions, including the periaqueductal grey, cingulate, and retrosplenial cortices. Additionally, longitudinal behavioural assessment reveals earlier sensorimotor symptoms in the BMT in R6/1 female at presymptomatic stage (8-w), while both sexes showed increased tolerance and reduced avoidance and approach responses at 12-w and 20-w.

These findings reveal that SC in HD has mHTT aggregation, synaptic deficits and disrupted connectivity, resulting in sensorimotor alterations with early onset and sex-dependent vulnerability. Understanding these changes provides crucial insight into HD pathophysiology and highlights SC dysfunction as a potential target for early therapeutic intervention.

Funded by Ministerio de Ciencia y Innovación and AEI (CNS2023-143999;PID2021-124896OA-I00;PID2020-119386RB-I00;PID2022-1363180B-I00), Horizon Europe (101129705) and Marie Skłodowska-Curie Actions (101104889).

Synaptic and anatomical insights into cortical modulation of the dorsolateral striatum

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The basal ganglia are a set of highly interconnected subcortical nuclei involved in motor control, among other functions. Dysfunction of the basal ganglia are directly associated with Parkinson's and Huntington's disease. Within the basal ganglia, the striatum is the main input nucleus. It contains diverse types of neurons including GABAergic projection, known as Medium Spiny Neurons (MSNs), as well as various types of interneurons.
The dorsolateral striatum (DLS) is considered part of the sensorimotor system, receiving primarily excitatory but also inhibitory transmission from cortical areas. Most of the studies aiming to understand these corticostriatal sensorimotor circuits and their related functions have focused on the primary motor (M1) and somatosensory (S1) cortices and their interactions with the DLS. However, axons from frontal association cortex (FrA), a putative premotor area, strongly innervate the DLS, yet their role in modulating DLS synaptic activity and functions remains unclear.
In this study, we aim to decipher the function of different sensorimotor cortices in the modulation of DLS activity. To this end, we first investigated synaptic transmission by the optogenetic activation of FrA, M1 and S1 cortical areas on MSNs in the DLS, using in vivo and ex vivo whole-cell patch-clamp recording. Our results revealed distinct synaptic dynamics stimulating FrA compared to M1 and S1. In order to clarify these results, we examined the striatal innervation of parvalbumin (PV+) and somatostatin (SOM+) cortical interneurons. Using viral tracers and transgenic mice, we selectively infected them in FrA, M1 and S1 and quantify their projections. We observed that PV+ and SOM+ M1 axons were distributed along the anterior DLS, while S1 axons were located mainly in the posterior striatum; on the other hand, interneurons projections from FrA were scarce. These results demonstrate a high specificity of cortical GABAergic innervation into the striatum.

The Effects of Aging on the Function of Ocular Cold Thermoreceptor Neurons

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Changes in corneal nerve morphology, density, and activity develop with aging. This study investigates how age affects the activity of corneal cold-sensitive trigeminal ganglion (TG) neurons, spontaneous blinking frequency, and basal tearing.

Background activity (BA) of ocular cold thermoreceptor TG neurons at basal corneal temperature, as well as their response (peak frequency -PF- and response duration) to temperature changes (induced by saline drops at varying temperatures), were recorded extracellularly with low-impedance tungsten electrodes in young (3-6 months) and aged (20-24 months) male Wistar rats. Spontaneous blinking was measured in awake rats, and basal tearing was assessed in young and aged isoflurane-anesthetized rats.

Based on their BA, cold TG neurons were classified into High Background (HB; BA > 5 imp·s-1) and Low Background (LB; BA < 5 imp·s-1). In aged animals, the BA of HB neurons was significantly lower ($p<0.05$), and the response to cold was significantly longer ($p<0.01$) compared to young rats. Additionally, there was no correlation between the PF and cold stimulus intensity in aged animals, particularly in LB neurons ($R2= 0.4296$, $p<0.05$ vs. $R2= 0.0437$, $p=0.47$; young and aged, respectively). Basal tearing was significantly higher in aged animals (13.2±3.4 vs. 32.1±5mm, $p<0.05$), whereas blinking frequency remained unaffected by age.

In summary, the spontaneous and cold-evoked activity of corneal cold-sensitive trigeminal neurons is altered with aging. These changes may contribute to the age-related reduction in cold sensitivity and modifications in tearing.

Trigeminal allodynia and microglial activation: Sex and estrous cycle-dependent differences

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Neuropathic pain after peripheral nerve injury is influenced by molecular and cellular mechanisms, with sex-related differences in behavioral dysfunction. Orofacial pain is more prevalent in females, making sex a key risk factor. To investigate genetic and hormonal contributions to sex differences in craniofacial neuropathic pain, we assessed facial mechanical allodynia in young adult CD1 mice of both sexes after unilateral chronic constriction injury (CCI) of the infraorbital nerve. Females in different estrous phases were included. Motor responses to Von Frey stimuli were tested bilaterally 12–14 days post-CCI. Both sexes exhibited mechanical allodynia on the operated side compared to the contralateral side and controls, with no overall sex differences. However, allodynia scores in females varied depending on the estrous phase at CCI induction. Females in diestrus had higher allodynia scores than males and females in other phases, whereas those in proestrus did not develop allodynia, suggesting a protective role of high estrogen levels against neuropathic pain. We also found sex-dependent differences in Iba-1-expressing microglia in laminae I-II of the caudal spinal trigeminal nucleus (Sp5c). While microglial activation was observed in both sexes, it was dependent on the estrous phase at CCI in females. Females operated at diestrus showed significantly increased Iba-1 expression compared to controls, whereas proestrus and estrus females did not. These findings highlight the role of hormonal fluctuations in neuropathic pain and microglial activation, providing insights into sex-specific mechanisms underlying orofacial pain.

Fundings: Agencia Estatal de Investigación, Spain, and Fondo Europeo de Desarrollo Regional (FEDER): PID2021-125039NB-I00; UAM – CAM, Programa de estímulo a la investigación de jóvenes doctores, Project: SI3-PJI-2021-00508.

Using Non-Invasive Brain Stimulation to Assess GABAergic and Glutamatergic Modulation During Cognitive Inhibition Tasks

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Paired-pulse transcranial magnetic stimulation (ppTMS) applied over the primary motor cortex (M1), combined with motor-evoked potential (MEP) recordings, provides a non-invasive approach to assess intracortical inhibition and excitation. Short-interval ppTMS (4 ms) reduces MEP amplitudes, reflecting GABAergic inhibition, whereas long-interval ppTMS (12 ms) increases MEP amplitudes, indicating glutamatergic facilitation.
In a previous study, we employed a ppTMS protocol to investigate how language comprehension modulates intracortical GABAergic activity. Twenty-two participants read action-related sentences in affirmative or negated forms (e.g., Ahora [sí] [no] abrirás la mochila), while single-pulse (baseline), short-interval, or long-interval ppTMS was applied over the left M1 at verb onset (abrirás). Results revealed that negated sentences, compared to affirmative ones, selectively modulated MEPs in the short-interval condition, suggesting GABA_A-mediated inhibition linked to negation processing. No significant effects were observed for non-action sentences, underscoring the role of inhibitory networks in action-related negation within M1.
Building on these findings, we conducted a new study using a ppTMS-EEG co-registration approach to obtain a more direct measure of intracortical inhibition and excitation. Participants completed the Hayling task, generating either a contextually expected word (association condition) or an unrelated word (inhibition condition). By analyzing EEG responses to single-pulse, short-interval, and long-interval ppTMS, we assessed GABAergic and glutamatergic modulation. Preliminary results indicate greater GABAergic inhibition in the response-inhibition condition compared to the association condition.
These findings demonstrate the utility of ppTMS in assessing inhibitory processes during cognitive tasks, with potential applications in studying inhibition-related disorders such as schizophrenia, OCD, ADHD, and depression.

Visual Impairment by Dopaminergic Depletion in the Mice Retina: Molecular and Histofunctional Manifestations

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Introduction: Parkinson's Disease (PD) is a neurodegenerative condition primarily associated with the loss of dopaminergic neurons. This degeneration occurs in the substantia nigra of the midbrain and extends to the retina, where Dopaminergic Amacrine Cells (DAC) play a critical role. In the retina, PD is linked to visual impairments, including motion perception deterioration. Retinal directional selective ganglion cells (DSGC), crucial for motion detection, are influenced by inhibitory inputs from Starburst Amacrine Cells (SAC). To explore this connection, this study used 6-hydroxydopamine (6-OHDA), a neurotoxin that induces dopaminergic neuron degeneration through oxidative stress and mitochondrial dysfunction. Our hypothesis posits that intravitreal 6-OHDA administration depletes retinal dopamine, impairing motion perception as a consequence of histological, molecular and functional changes. The research aimed to characterize these effects by investigating the interplay between DAC and SAC, as well as broader retinal alterations, to better understand the mechanisms underlying visual dysfunction in PD.

Material and methods: this study examined the effects of 6-OHDA-induced dopaminergic depletion on retinal function in C57BL/6J mice. Behavioral tests (optomotor, water maze), electroretinography, and molecular analyses were performed pre- and post-injection. In addition, immunohistochemistry and confocal imaging explored the retinal connections between DAC and SAC to understand the mechanisms of visual dysfunction linked to Parkinson's disease.

Results: fifteen days after 6-OHDA treatment, mice exhibited significant reductions in motion perception, visual acuity, contrast sensitivity, and electroretinographic responses. After 30 days, molecular analysis revealed increased concentrations of dopamine metabolites. Additionally, retinal dopamine degeneration was reasserted by immunohistochemical staining of synaptic contacts between DAC and SAC.

Conclusion: this research reveals the appearance of visual and retinal alterations after 6-OHDA administration. The results suggest a promising applicability of this experimental model in the study of dopamine functions in the retina, as well as the pathophysiological mechanisms of PD and the development of new therapeutic strategies.

COGNITIVE AND BEHAVIORAL NEUROSCIENCE

INDEX

"Harmonizing the Brain: The Impact of Music on Children with Neurological Disorders"

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Background
Music is increasingly recognized as a powerful neuromodulatory tool with significant implications for cognitive and emotional development. Recent research underscores its potential in neurorehabilitation, particularly for children with neurological disorders affecting language processing, motor function, and overall cognitive abilities. While accumulating evidence supports the efficacy of music-based interventions, the underlying neural mechanisms remain insufficiently understood. This study aims to elucidate the effects of an intensive music training program on cognitive, linguistic, and neurophysiological functions in children with severe neurological impairments, with a specific focus on prosodic processing and neural plasticity.
Materials and Methods
A total of 20 children (ages 7–17) diagnosed with neurological disorders impacting language processing participated in a structured 4- to 8-month music intervention program. The training included rhythm exercises, vocal modulation, and instrumental engagement, specifically designed to enhance sound parameter perception relevant to prosody.
Pre- and post-intervention cognitive and linguistic assessments were conducted using standardized neuropsychological batteries. Functional magnetic resonance imaging (fMRI) was employed to assess structural and functional changes in neural circuits involved in auditory and language processing.
Results
Post-intervention assessments demonstrated significant improvements in language processing and auditory discrimination compared to baseline. fMRI analyses revealed increased activation in Broca's area, and the cerebellum, indicative of enhanced neural plasticity. Notably, these improvements exhibited a dose-dependent relationship with the duration of the musical training.
Conclusions
These findings provide compelling evidence that structured music-based interventions promote neuroplasticity and cognitive-linguistic enhancement in children with neurological disorders. The observed neural and behavioral changes underscore the therapeutic potential of music in pediatric neurorehabilitation. Further longitudinal studies are warranted to investigate the durability of these effects. Music therapy, when integrated as an adjunct to conventional treatments, represents a non-invasive, engaging, and highly promising approach for improving neurodevelopmental outcomes in children with neurological impairments.

A Neural Circuit Integrating Cortical and Hypothalamic input to Drive Courage in Aversive Environments

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The ability of animals to adaptively select behavioural responses to environmental changes is essential for survival. These decisions rely on context, internal state, and prior experience. The ventral lateral geniculate nucleus (vLGN), an inhibitory prethalamic nucleus, plays a key role in controlling defensive behaviors, with its activity shaped by prior experience and threat expectation (Fratzl et al.; Mederos et al.). We hypothesized that the vLGN is a critical hub for selecting behavioral strategies, such as balancing predator avoidance and foraging for resources. And, indeed, using optogenetics and fiber-photometry in freely behaving mice, we found that vLGN activity can dictate decisions regarding whether to stay in 'safety' or enter potentially aversive environments, such as the open arms of an elevated plus maze or leaving a shelter after experiencing an innately threatening visual stimulus. Next, we sought to determine inputs that control these decisions through vLGN pathways, and found that both cortical and subcortical systems converge in the vLGN to influence behavioral strategies. Input from retrosplenial cortex and from the ventromedial hypothalamus have opposing effects on the animal's decision to leave safety, which is also reflected in the information these projections convey to vLGN during natural behaviour. Our preliminary data suggests that these inputs are integrated via a local inhibitory vLGN circuit.
These findings indicate that the vLGN integrates signals for assessing threat versus safety, as well as the animal's intentions to regulate anxiety-related avoidance behaviors and to facilitate risky behaviours in threatening environments.

Astrocyte NMDA receptor differentially modulates aversive and reward learning

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The primary motor cortex (M1) plays an essential role in instrumental and classical conditioning requiring neuronal NMDA receptor-dependent neuronal plasticity. It is, however, not known what role the M1 astrocyte NMDA receptor plays in these two types of associative learning. We hypothesized that astrocyte-NMDAR-dependent gliotransmission might control learning through intimate communication with neuronal circuits. Selective removal of the M1 cortex NMDA receptor impaired negatively reinforced trace eyeblink conditioning and learning-dependent synaptic strength. Surprisingly, it enhanced the positively reinforced instrumental conditioning. Our results suggest that the primary somatosensory projections to the M1 synapse and from the ventral tegmental area to the M1 synapse cooperate with astrocyte NMDA receptor-dependent gliotransmitters to differentially modulate associative learning. Our study provides crucial insight into the role of astrocyte NMDA receptor that could be relevant for neurological and psychiatric diseases.

Astrocyte-Neuron signaling in Dorsal Raphe modulates social behavior

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Social behaviors require the cooperation of multiple brain areas in order to integrate information coming from sensory cues and internal states (Chen and Hong, 2018). Brain areas such as the dorsal raphe nucleus (DR) and medial prefrontal cortex (mPFC) have been linked to several aspects of social behaviors, including social recognition, social reward, empathy-like behaviors, social isolation and social dominance, among others.
Astrocytes play crucial roles in brain homeostasis. As regulatory elements of neuronal and synaptic physiology, astrocyte activity also has an impact in brain circuits that control animal behavior (Oliveira et al. 2015). However, their contribution to social behaviors is yet poorly explored (González-Arias et al. 2023).
Given the high interconnectivity of the social brain, we investigate whether the astrocyte-neuron signaling in the DR can impact the actions of DR neurons in their targeted areas, such as mPFC, and how DR astrocytic activation can modulate different aspects of social behaviors. To answer those questions, we combined optogenetics and electrophysiological recordings, both ex vivo, and in vivo during social behavioral paradigms.
We found that stimulation of DR astrocytes reshaped social preference and promoted social bonding. This phenomenon was further accompanied by changes in mPFC neural activity during social explorations, showing increased social identity encoding and enhanced activity towards the bonded subject. At the cellular level, DR astrocytic stimulation increased the excitability of DR->mPFC projecting neurons ex vivo.
These data support the ability of astrocytes to modulate of long-range circuits that control social behavior.

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Bacterial and immune communities of the gut: Their role in depression

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Depression is a heterogenous neuropsychiatric disease, and one of the leading causes of disability worldwide, affecting 280 million people as stated by the World Health Organization. Current treatments for depression are ineffective in a great number of patients and the search for alternative treatments is an essential focus for research. Utilization and/or modification of the microbes that populate the intestine is emerging as a new potential avenue to treat psychiatric disorders. In addition, the crosstalk between intestinal bacteria and different immune components seems to have a key role on mental health as well.

We performed a metagenomic analysis on the microbiota present in three regions of the mouse gut: proximal (1) and distal (2) regions of the small intestine and colon (3). We found that community composition was different in all three of them. Furthermore, when the microbial populations from those regions were introduced in microbiota-depleted mice, we found that animals who were given the bacteria corresponding to regions 2 and 3 were able to recover their original microbiota. Interestingly, those given bacteria from region 1 were unable to do that, and showed more depressive symptoms compared to those treated with microbiota from regions 2 and 3. Moreover, levels of inflammatory markers showed that treatment with Regions 2 and 3 also lead to a bigger immune response, while treatment with region 1 showed a similar response to the untreated control.

These results might serve to identify new therapeutic immune targets and bacterial combinations to treat depression and reduce the clinical and societal burden of this psychiatric condition.

Balancing focus and distraction: the role of the superior colliculus in visual attention and distraction resilience

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Distractor suppression is crucial for controlling visuospatial attention. Both cortical and subcortical areas contribute to this process, with the ancient midbrain region of the superior colliculus (SC) playing a central role. The SC houses core orienting circuits and serves as an integration node for brain-wide cortical (visual, parietal, and frontal), subcortical (e.g. nigral, parabigeminal), and multisensory (visual, auditory, and somatosensory) inputs. Data from our lab in freely-moving mice suggest that disinhibition of collicular vGAT+ neurons reduces visuospatial distractor resilience without disrupting goal-dependent behavior. To gain a mechanistic, circuit-based understanding, we have developed a head-fixed visuospatial attention-distractor behavioral task to decode SC E/I activity using: 1) high-density Neuropixels recordings, 2) cortical LFP recordings to assess attentional and arousal states, along with eye movement and pupil dilation, and 3) opto/chemogenetic interrogation of long-range visual and cortical inputs targeting inhibitory (vGAT+) and excitatory (vGluT2+) neurons in the lateral division of the intermediate SC (l.SCi). We anticipate this study will provide valuable insights into the mechanisms underlying distractor alert-to-suppress processes.

BOLD Correlates of Learning and Memory: An fMRI Study in Behaving Mice

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Learning involves adapting to environmental contingencies by forming stimulus-value associations. We investigated sensory discrimination learning in mice using a reversal GO/NOGO task while recording BOLD activity with fMRI. By analyzing brain-wide activation across training, we identified learning-dependent neural recruitment and task-specific processing differences between GO and NOGO trials.

Mice (n=16) performed a head-fixed GO/NOGO task while undergoing fMRI (Bruker BioSpec 7T). Stimuli and rewards were delivered via Arduino-controlled air and water valves, and lick behavior was recorded using MRI-compatible video. The GO stimulus (left-side air puff) preceded water reward (500 ms delay), while the NOGO stimulus (right-side air puff) was never reinforced. Upon learning (6 sessions of 60 trials each), the rule reversed (8 mice, 3 extra sessions). Functional images were acquired using a gradient-echo EPI sequence (TR = 2 s, TE = 16 ms, 22 slices, resolution = 0.125 mm × 0.125 mm × 0.5 mm). Data were preprocessed in FSL Statistical analysis used FEAT (FSL) with a GLM approach, employing a double-gamma hemodynamic response function (HRF) and a z-stat threshold of 2.6.

Mice progressively increased anticipatory licking during GO trials, confirming successful learning. At early training stages, BOLD activity was localized to the primary somatosensory cortex (S1), with right S1 responding to GO and left S1 to NOGO. After learning, GO trials recruited broader brain regions, likely reflecting reward-related and decision-making processes, whereas NOGO trials remained confined to S1. During reversal learning, mice exhibited both behavioral and neural adaptations, indicating memory updating and response flexibility.

We are currently using optogenetics to investigate the role of inhibitory basket cells in the dentate gyrus (DG) of the hippocampus in reversal learning. We hypothesize that silencing these neurons will facilitate faster behavioral flexibility in this sensory discrimination task.

Boosting statistical learning by weakening the dorsolateral prefrontal cortex: a noninvasive brain stimulation study

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Learning probability-based regularities, i.e., statistical learning, is crucial for adaptive behaviour, skill acquisition, and habit formation. This model-free process is thought to compete with model-based processes, which rely on the dorsolateral prefrontal cortex (DLPFC). While previous research suggests that weakening the bilateral DLPFC can enhance statistical learning, the hemisphere-specific effects of this modulation remain unclear. Here, we investigated whether weakening the DLPFC by low-frequency repetitive transcranial magnetic stimulation (rTMS) affects statistical learning. We hypothesized that weakening the DLPFC would boost statistical learning. We also expected the left stimulation to drive this effect, due to different strategies linked to the left and the right DLPFC. Ninety-five participants were assigned to one of four groups receiving sham stimulation or 1 Hz (inhibitory) rTMS over the left, right, or bilateral DLPFC. Participants completed a probabilistic sequence learning task for five times five minutes. In the breaks between blocks, they received rTMS or sham stimulation. Results showed that the right and bilateral rTMS groups outperformed the sham group, with the right DLPFC group also exceeding the left DLPFC group.
These findings are in line with studies showing better model-free functioning when the DLPFC is weaker. However, instead of the left DLPFC inhibition driving statistical learning improvements, the right DLPFC plays a more critical role. This may be due to functional connections between the DLPFC, striatum, and motor cortices. Our findings support the competition theory and highlight the importance of considering hemispheric differences in the neural mechanisms underlying statistical learning.

Calorie restricted male rats learn value-based decisions faster

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There is strong evidence that moderate caloric restriction (CR) has beneficial effects on cognition, including learning and memory. Most of this evidence comes from studies using relatively simple behavioral tasks, such as the Morris water maze. To test whether this advantage generalizes to decision-making and whether this effect depends on sex, we separated female and male rats into two groups: ad libitum (AL), which had unlimited access to food; and CR, which could eat 70% of their daily food intake. After at least 6 weeks under these conditions, we tested both groups in a value-based task. Briefly, rats had to go through a series of nose pokes and learn, by trial and error, which of the two response alternatives was optimal (rewarded 80% of the times) and which one was non-optimal (rewarded 20% of the times); after 6 consecutive optimal responses, the optimal alternative was switched and a new learning episode started. We found that CR male rats learned the task significantly faster than AL males, while females showed no significant differences. Once they know the rules of the task, their maximum performance is similar across feeding conditions. By elucidating the underlying mechanisms and potential benefits of CR, we hope to contribute to the growing body of evidence supporting dietary modulation as a viable strategy for promoting cognitive health and longevity.

Can the Enhancement of Midfrontal Theta Decrease Predictive Learning?

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Midfrontal theta oscillations (4–8 Hz) are typically linked to cognitive control, conflict monitoring, and adaptive learning. Enhanced midfrontal theta could theoretically shift learning strategies from model-free, predictive processing-based approaches to more controlled, rule-based ones. Predictive learning relies on detecting statistical regularities and making probabilistic inferences, often benefiting from implicit, model-free mechanisms. A key question arises: if we entrain endogenous midfrontal theta oscillations, which, in turn, increases cognitive control and top-down interference, might it reduce reliance on predictive learning by enforcing model-based processes or suppressing spontaneous model updates?
To address this question, we applied 2 mA theta transcranial alternating current stimulation (tACS) at 6 Hz for 20 minutes to healthy adults over the bilateral frontal cortices. Participants completed an eye-tracking version of an implicit probabilistic sequence learning task during stimulation, which assesses statistical learning through anticipatory responses. We compared the statistical learning performance of the active tACS group with a sham stimulation group. Our results show decreased statistical learning performance during and shortly after stimulation in the active tACS group compared to the sham group. However, anticipatory eye movements revealed no difference between the groups. Supporting the competition theory, our findings suggest a causal relationship between midfrontal theta and statistical learning, as entrainment of the frontal cortices at 6 Hz mitigated statistical learning. A deeper understanding of these competitive cognitive processes may lead to new methods for enhancing learning and memory functions.

Classically conditioned eyelid and vibrissae responses, in mice, are related to motor cortex neurons, but not originated in them

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Classical eyeblink conditioning is one of the most widely used experimental models for the study of the neural mechanisms underlying the acquisition of new motor and cognitive skills in behaving mammals. Currently, some studies pointed to the motor cortex, the cerebellum, or other areas, as the structures putatively responsible for this type of learning. To determine the specific contribution of the motor cortex to the generation of conditioned eyelid responses, and knowing the existing overlap between eyelid motor and vibrissae neurons in the motor cortex of these animals, we studied the temporal correlation between the unitary activities of the identified eyelid motor cortex and vibrissae neurons, the electromyographic activity of orbicularis oculi and vibrissae muscles, and eyelid positions, during classical conditioning of eyelid and vibrissae responses, using delay and trace conditioning paradigms in behaving mice. Mice were prepared for classical conditioning of eyelid and vibrissae responses and for recording of unitary activity of motor cortex neurons. Chronic electrodes for stimulation and recording were implanted in the eyelid and vibrissae muscles, and a craniotomy was performed in the cerebellar skull. Eyelid movements were recorded with the aid of a magnet attached to it. Motor cortex neurons were identified by their antidromic activation from the ipsilateral red nucleus or the contralateral facial nucleus. The involvement of motor cortex neurons in reflexively evoked eyelid responses and the kinematic and oscillatory properties of eyelid movements evoked by microstimulation of the motor cortex were also studied. Results show the involvement of the motor cortex in the execution of elicited conditioned responses during the classical conditioning task. However, a temporal correlation analysis showed that both electromyographic activities preceded the firing of motor cortex neurons, so they must be more related to the reinforcement and/or correct execution of the conditioned responses than to their acquisition and storage.

Compulsivity and Alcohol Abuse: Sex-Specific Molecular and Metabolomic Alterations in a Preclinical Model

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Compulsivity refers to the inability to stop a persistent behavior that has become inappropriate for the given circumstances. Clinical research indicates a comorbidity between alcohol use disorder and obsessive-compulsive symptomatology, leading to a pattern of compulsive alcohol consumption. Thus, compulsivity may be a vulnerability factor contributing to loss of control and alcohol abuse. The aim of this study was to identify biomarkers associated with vulnerability to compulsive alcohol drinking in both sexes. Male and female Wistar rats were exposed to a preclinical compulsivity model known as Schedule-Induced Polydipsia (SIP), initially with water and the with alcohol; and distinct groups were formed based on their levels of water and alcohol intake. Following group formation, NMR metabolomic analysis was conducted on fecal samples from the final SIPwater and SIPalcohol sessions, focusing on key metabolites such as tryptophan and tyrosine that are essential for neurotransmitter synthesis. Finally, gene expression was also evaluated using RT-qPCR on frontal cortex tissue samples to analyze the expression of various markers (related to neuroplasticity and serotonergic, glutamatergic, and GABAergic systems). The cluster analysis based on water and alcohol consumption led to the formation of four groups: low compulsive, compulsive alcohol, compulsive water, and high compulsive. The results will be analyzed in terms of changes in gene expression and the metabolomic profile associated with these distinct consumption patterns. Gene expression analysis will identify the molecular pathways involved, while metabolomic analysis will uncover biochemical changes associated with compulsive alcohol drinking. In conclusion, the identified consumption patterns provide a framework for exploring the molecular and biochemical factors associated with compulsive alcohol drinking behavior.
Funding: I have received a scholarship from the JA. PID2022-139286NB-I00 PGC, MCIN/AEI/10.13039/501100011033; PND-2022I024 PNSD, MISAN; SUBV23/00027, MIC, DGOJ, Gobierno de España and Fondos Feder; and PPIT-UAL, Junta de Andalucía-ERDF 2021-2027. Objective RSO1.1. Programme: 54.A.

Decoding cognitive resilience in aging and multiple sclerosis: a transcriptomic approach

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Cognitive resilience (CR) refers to maintaining cognitive function despite aging or disease-related changes. Identifying CR molecular signatures could offer insights into neuroprotection in aging individuals and people with Multiple Sclerosis (pwMS). We hypothesize that CR biomarkers in cognitively preserved healthy individuals and pwMS will share mechanisms supporting cognitive function. This study aims to identify CR biomarkers in cognitively preserved individuals and pwMS with cognitive impairment. Transcriptomic analysis (RNA-seq) was performed on buffy coat samples from three cohorts: healthy individuals (60-90 years) and pwMS (60-64 years) with preserved cognition, and pwMS (60-64 years) with cognitive impairment, defined as scores <7 points. Statistical comparisons using multivariate and correlation analyses. Differential gene expression (DEGs) and pathway enrichment identified molecular processes linked to CR. A total of 6,048 differentially expressed genes were identified, with 3,397 downregulated and 2,651 upregulated. Key findings showed mitochondrial gene expression differences (e.g., MT-ND4L, MT-ND1), indicating dysfunction in the electron transport chain in pwMS. Downregulated genes related to cell division and genomic stability (e.g., ASPM, TROAP) suggested alterations in cellular regulation. Enriched pathways included oxidative phosphorylation and ATP production, pointing to bioenergetic deficits. Seventy-one genes associated with MS pathology were common in both cognitively preserved and impaired individuals with MS, with specific genes (e.g., SSBP2, PRR7, RTN2) differentially expressed in those with preserved cognition. Preliminary analysis of cognitive functions and executive-attentional abilities revealed overexpression of mitochondrial genes (e.g., MT-ND4L, MT-TW) and underexpression of genes related to cellular stability (e.g., TROAP, SPTA1). The findings suggest that mitochondrial dysfunction and altered gene regulation contribute to cognitive impairment in pwMS, while specific gene expressions in cognitively preserved individuals highlight resilience mechanisms. The study underscores mitochondrial dysfunction and bioenergetic alterations as key factors in MS and aging, with identified biomarkers offering insights into strategies for enhancing CR and slowing cognitive decline.

Diet Modulates Cognitive and Motor Impairments in a TDP-43 Proteinopathy Murine Model

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Diet plays a critical role in modulating the metabolic dysfunctions associated with TDP-43 proteinopathies, including ALS and FTD. Emerging evidence from mouse models of TDP-43 proteinopathies suggests that dietary interventions can affect key pathwayssuch as lipid metabolism, mitochondrial function and glucose utilisation. Studies suggest that dietary strategies, including caloric restriction and high-fat diets, may not only modify TDP-43 mislocalisation and aggregation, but also improve the cognitive and motor impairments observed in these models. For example, high-fat diets have been shown to improve motor function, while caloric restriction may reduce neuroinflammation and oxidative stress, thereby preserving cognitive abilities. Despite its therapeutic potential, the impact of diet on TDP-43 pathology and associated cognitive and motor déficits remains underexplored. We hypothesise that different types of diet may selectively modulate the phenotypic characteristics of TDP-43 proteinopathies, potentially influencing disease onset, progression and severity.

We performed a comprehensive study using a mouse model carrying a point mutation in the Tardbp gene (TardbpM323K/M323K), which develops cognitive and motor impairments along with cytoplasmic mislocalisation of TDP-43. Mice were subjected to three different diets from 6 weeks to 12 months of age. We longitudinally evaluated the effects of these diets on disease progression using motor and cognitive tests and analysed protein expression using Western blot and immunohistochemistry, including TDP-43 mislocalisation in the nervous system. We also took a functional approach by performing brain metabolism studies using 18F-FDG PET.

Caloric restriction demonstrates a more pronounced improvement in cognitive function, while a high-fat diet provides modest benefits in motor performance, suggesting distinct yet complementary mechanisms by which dietary interventions influence neurodegenerative processes.

This study provides a foundational framework for understanding how dietary interventions can impact brain function and modulate pathological changes induced by genetic mutations. These findings have significant implications for developing metabolic-based therapeutic strategies in neurodegenerative diseases.

Dissecting reward and aversion processing populations in the basal amygdala-nucleus accumbens pathway

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The basal amygdala (BA)–nucleus accumbens (NAc) pathway plays a crucial role in processing rewarding and aversive stimuli. However, the specific neuronal populations involved in these two processes remain to be fully understood. This study aims to dissect the contributions of distinct BA-NAc neuronal subpopulations, particularly focusing on cholecystokinin-expressing (CCK+) and non-CCK (CCK−) glutamate neurons and their synaptic connections with medium spiny neurons (MSNs) in core and shell regions of the NAc. To achieve this, we have employed a combination of anatomical tracing, genetic targeting, and functional analysis techniques.

Firstly, we quantified the number of BA-NAc projection neurons from medial, lateral, and ventral BA divisions to NAc core and NAc shell regions using retrograde cholera toxin subunit B (CTb) and anterograde adeno-associated viruses (AAVs), also studying the specific contribution of CCK+ and CCK- neurons to the BA-NAc pathways. We refined our targeting strategy by employing Cre-Off and Cre-On AAV-based eGRASP approaches to quantify synaptic connections between BA-NAc CCK+ and CCK− neurons and NAc core and NAc shell dopamine D1 receptor-expressing MSNs (D1 MSNs), combined with laser capture microdissection and RT-qPCR to assess specificity.

This comprehensive approach will elucidate the functional organization of BA to NAc pathways, shedding light on how distinct neuronal populations contribute to reward and aversion processing. Understanding these mechanisms could have significant implications for neuropsychiatric disorders characterized by maladaptive reward and aversion responses, such as depression.

Do auditory novel stimuli evoke cortical state changes?

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Long-latency responses to unexpected deviant stimuli occur in different cortical states, and are often attributed to attention, arousal or behavioural choice. Cortical slow oscillations occur during sleep or anesthesia and consist of synchronised changes between “Up” and “Down” phases throughout the cortex, corresponding to high- and low-activity levels. Sensory stimuli can evoke cortical Up phases, but whether this occurs in the oddball paradigm is unknown. We implanted Long-Evans female rats with ECoG arrays placed bilaterally over auditory and medial prefrontal cortex. Recordings were made under urethane anaesthesia, an established model of Up/Down phases. Auditory stimuli consisted of a classical oddball paradigm using a frequency pair of 10 and 14.142 kHz or omissions. We identified Up phase initiations as peaks greater than 3 SDs in the LFP traces from medial prefrontal cortex with no peaks in the preceding 1.5s. We observed spontaneous cortical slow oscillations, consisting of “Up” and “Down” phases. Up phases occurred after most deviant stimuli, with a ~200 ms latency in the prefrontal cortex. Synchrony analysis revealed a significant temporal relationship between deviant and Up phase initiations, suggesting that Up phases were evoked by both ascending and descending deviants. In one animal, deeper anesthesia increased the percentage of deviants evoking Up phases, after further doses, this effect reversed due to difficulty exiting the Down phase. Unlike deviants, omissions did not initiate Up phases. Our results show deviants may evoke cortical state changes, seen as long-latency responses. These findings suggest that state changes could underlie previously observed long-latency deviant responses. How these state changes correspond to attention, arousal or behavioural choice during awake oddball paradigms awaits future studies.

Financial support was provided by AEI (grant #PID2023-148541OB-I00) to M.S.M.; MCSA-USAL4EXCELLENCE (grant #PROOPI-340) to M.S.M. and A.H.; JCYL (SA218P23) to MSM. L.H.B. held a fellowship PREP2023-148541OB cofounded by MCIN/AEI/10.13039/501100011033 and FSE+.

EFFECT OF LEFT-DLPFC TRANSCRANIAL STATIC MAGNETIC STIMULATION (tSMS) AND GAIT-INDUCED FATIGUE ON THE RISK OF FALLING WHILE DUAL-TASK WALKING IN HUMAN AGEING

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Gait-induced fatigue might increase fall-risk during human walking and there is also compelling evidence that dual-task walking increases fall-risk, especially in elder people. However, the interaction effects between gait-induced fatigue and dual-task execution on fall-risk is un-known. We evaluated this interaction by asking 12 elder and 12 young healthy participants to walk for up to 2h and 20min. over a treadmill, at their preferred velocity, in sets of 5min. At the end of every set, fatigue perception was assessed with a visual analogue scale (VAS). Participants were informed the walking-test would end whenever they wished. The CV (%) of stride-cadence (indicator of falling risk) was computed every set. The two initial and final walking-sets were performed concurrent with two tests of different cognitive load, the Stroop or a Word-Reading test.
To understand a putative role of the left-DLPFC in the relation between fatigue development, dual task execution, and the CVstride-cadence, participants walked over the treadmill in two different sessions (>1wk apart). One session, their left-DLPFC was stimulated with real-tSMS, the other day was sham-tSMS; sessions counterbalanced in order. In the second session, participants were asked to walk the same number of set than the first one.
Fatigue developed along the sessions (a moderate increase of ≈4 VAS points, $p<0.001$), regardless real/sham tSMS and for both groups, but elder participants completed less sets. CVstride-cadence was larger for elder participants ($p<0.001$) the two sessions. However, fatigue development produced no effect on CVstride-cadence, and this was not different for elder and young participants, real or sham tSMS, and sets executed with different levels of cognitive load.
In conclusion, moderate levels of gait-induced fatigue have no effect on CVstride-cadence. tSMS of the left DLPFC has no effect on gait-induced fatigue development. tSMS of the left-DLPFC has no effect on CVstride-cadence.
Funding: Plan Nacional MINECO-DEP2017-87601-R

Effects of adolescent alcohol and cannabinoid exposure on age-related cognitive decline in a rat model

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Alcohol and cannabis are among the most commonly used substances, frequently consumed together, with a higher prevalence among men, particularly during adolescence; a critical period for brain development This study investigates the long-term impact of adolescent exposure to these substances on age-related cognitive decline in male and female rats. Sprague-Dawley rats (n=10 per group) were exposed to alcohol or Δ-9-tetrahydrocannabinol (THC) every three days for one month, starting on postnatal day 35. One group consumed an alcohol solution (5% v/v in water) for 24 hours, another received THC (1.5 mg/kg, i.p.), and a third group was exposed to both substances. Cognitive function was assessed using the Barnes maze and the eight-arm radial maze to evaluate learning and visuospatial memory, while motor coordination was tested using the rota-rod. Behavioral assessments conducted immediately after the treatment period revealed no significant differences between groups. However, at 15 months of age, female rats from all treated groups showed a higher reduction of latency time to find the scape box during training and in some test phase; suggesting a better learning process and visuo-spatial memory. The same effect but less pronounced was found in males. However, alcohol-exposed males showed significantly improved performance in the reverse phase of the Barnes maze, suggesting enhanced cognitive flexibility. Additionally, alcohol-exposed males outperformed controls in the radial maze, indicating a potential protective effect against age-related working memory decline. In females and in the rota-rot test a similar effect was observed, an improvement of motor coordination by alcohol, an effect not observed in males. These findings suggest that adolescent exposure to alcohol and cannabinoids does not negatively impact on age-related cognitive decline. Instead, a subtle protective effect was observed, with notable sex differences. Funding: Plan Nacional de Drogas (Ministerio de Sanidad), Ref. 2021/022. M. Jiménez-García was supported by FPI_062_2021 grant.

Effects of Chronic Treatment with STX64 in R6/1 Mouse Model of Huntington's Disease (HD)

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Huntington's disease (HD) is an inherited rare neurodegenerative disorder caused by a mutation in the gene encoding the huntingtin (HTT) protein on chromosome 4. This mutation involves an abnormal expansion of CAG trinucleotide repeats in the DNA encoding the amino acid glutamine, leading to the production of an abnormally long version of the huntingtin protein, causing its abnormal folding and pathological accumulation.

Current treatments for Huntington's disease focus primarily on symptom management, as there is no cure or treatment that alters the course of the disease. It has been described that the inhibition of steroid sulfatase could alter the metabolism of neuroactive steroids by preventing the desulfation of steroid sulfates.

Inhibition of STS has been shown to increase longevity and improve protein aggregation, which is characteristic of diseases such as Alzheimer's, Parkinson's, and Huntington's. Aging studies in C. elegans have shown that treatment with STX64 increases the lifespan of this model. STX64 has also been tested in C. elegans models of Alzheimer's, Huntington's, and Parkinson's diseases. In all three cases, treatment with STX64 improved mobility and reduced protein deposits characteristic of these diseases compared to vehicle-treated models. Additionally, chronic oral administration of STX64 in advanced-stage Alzheimer's mouse models improved cognitive deficits and reduced amyloid deposition compared to vehicle-treated animals.

This study evaluates the impact of chronic treatment with STX64 in a transgenic murine model of Huntington's disease (R6/1 mouse). Various cognitive and behavioral tests were conducted to evaluate mice cognitive and motor function progression.

Results obtained indicate that chronic treatment with STX64 could have a beneficial effect in mitigating some symptoms of the disease, particularly at the cognitive and muscle strength levels. These effects could be gender dependent due to hormonal differences of each sex background.

Effects of mental fatigue on human postural balance and fall risk. Preliminarly results and discussion of a future protocol

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There is growing evidence that executive areas exert top-down control on basic motor functions and available data indicate that mental fatigue might impair balance, increasing the risk of falls. However, it is unclear if mental fatigue induced by sustained cognitive tasks, bound to different executive functions, has different effects on postural balance. Our hypothesis is that fatigue generated by cognitive tasks will increase balance alterations depending on the kind of cognitive task.
We tested if mental fatigue, subjectively evaluated with a visual analogue scale (VAS), has an impact on balance. Cognitive tasks inducing fatigue were Stroop-colour interference (focused on inhibitory control), N-back (focused on working memory), and Word-Reading (as control condition). We tested each cognitive task in a different session (9 sessions, in 3 young human participants, ongoing). We first tested baseline fatigue perception (VAS) and then postural balance (over a balance platform) with open and closed eyes, randomized in order; these are standard conditions for testing postural instability (closed eyes produces larger postural instability). Next, we tested balance during the execution of a cognitive task (different each day). The cognitive task remained continuous for 8 min, and balance was retested during the final minute of cognitive task execution. We finished with VAS retesting.
There was an evident increase in the postural sway (fall-risk indicator) at the end of the 8 min of the cognitive task, which was marker for Stroop and N-back than for Word-Reading; fatigue perception also developed more for Stroop and N-back than for Word-Reading.
Mental fatigue has an impact on postural stability. Fatigue induced by different cognitive tasks appears to have a different effect on postural balance. A future protocol for testing neurophysiological correlates of these effects will be discussed.
Funding: Plan Nacional PID2023-147447OB-I00

Effects Of Particulate Matter Exposure In An Aged Mouse Model Vulnerable To Cognitive Decline

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Apolipoprotein E4 (APOE4) is known as one of the strongest genetic risk factors for late-onset Alzheimer's disease, as it is associated with increased susceptibility to neurodegeneration. Carriers of the ApoE-ε4 allele exhibit greater neuroinflammation, oxidative stress, and impaired synaptic plasticity, all of which contribute to cognitive decline. Environmental factors such as air pollution, particularly exposure to particulate matter (PM), have been linked to the exacerbation of these pathological processes.

This study aimed to assess the effects of PM exposure on anxiety, learning, and memory in subjects genetically susceptible to neurodegeneration, and to evaluate the role of sex in modulating these factors. Thus, fourteen-month-old transgenic APOE4 mice, both male and female, were assigned to two experimental groups: sham and PM. Mice in the PM-exposed group received subchronic treatment based on exposure to PM in whole-body chambers for four weeks five days a week with a dose of 200 µg/kg/day, while sham-exposed mice underwent the same handling and chamber conditions to control for potential stress effects unrelated to PM exposure. Following treatment, behavioral tests in an Open Field and a Morris Water Maze (MWM) were conducted to evaluate exploratory behavior, anxiety, learning and memory.

Results revealed sex differences in cognitive function, memory, and anxiety-like behaviors in aged APOE4 mice. Although PM is known to contribute to neurodegeneration, its effects were only observed at a single time point in MWM retention, while the primary differences in this study were between males and females, particularly in long-term retention, rather than between exposure groups. These results suggest that sex may play a crucial role in modulating cognitive performance and susceptibility to environmental factors in ApoE-ε4 carriers. Understanding these inherent differences is essential for developing targeted strategies to mitigate cognitive decline in genetically at-risk populations.

Effects of reward on emotion recognition in Parkinson's Disease

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Dopamine plays a key role in both reward and emotions through fronto-striatal and limbic-striatal circuitries. Yet, how reward influences emotional processing is unknown. Parkinson's Disease (PD) is characterised by motor and non-motor symptoms associated to dopaminergic degeneration, including deficits in facial emotion recognition . Also, negative emotion detection is impaired in PD. These deficits are somewhat independent of cognitive decline, and may share underlying neural mechanisms, such as greater mesocorticolimbic dopaminergic pathway damage as the disease progresses. We aim to characterize how dopamine modulates the interaction between reward and emotions in patients with PD. Here, we used an incentivised static facial emotion recognition task in 17 PD patients while on and off medication. Behaviour and eye-tracking were employed to measure fixations, saccades, and pupil dilation upon high and low reward cues (sounds) followed by emotional categorization of faces. Repeated measures ANOVA on emotion recognition accuracy and reaction times revealed no significant main effect of reward condition ($F(1, 16) = 1.210$, $p = 0.286$) or medication ($F(1, 16) = 0.271$, $p = 0.609$). Yet, emotion recognition accuracy showed a trend indicating an interaction between medication state and reward condition ($F(1, 16) = 4.290$, $p = 0.053$). This preliminary finding suggests that the effect of reward condition (high vs. low), on emotion identification accuracy may differ depending on medication state (on vs. off). PD patients off medication had higher accuracy in the low reward condition (mean for high reward-off medication = 0.608; mean for high reward-on medication = 0.597; mean for low reward-off medication = 0.621; mean for low reward-on medication = 0.556). In the off medication state, patients with PD revealed enhanced emotion recognition accuracy, particularly in low-reward contexts, possibly indicating that dopaminergic medication can somehow disrupt emotional processing, supporting the dopamine 'overdose' hypothesis.

Effects of Transcranial Static Magnetic Stimulation on the Left Dorsolateral Prefrontal Cortex in Higher-Order Cognitive Functions: Randomness in Response Generation, Creativity, Decision-Making, and Risk-Taking

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Transcranial static magnetic field stimulation (tSMS) is a neuromodulation technique with inhibitory effects on brain activity. While its impact on motor, somatosensory, and visual cortices has been studied, its effects on higher-order cognitive functions remain unclear. This study investigated the influence of tSMS applied to the left dorsolateral prefrontal cortex (DLPFC) on randomness in response generation, creativity, decision-making, and risk-taking. One hundred four healthy participants were recruited across three double-blind, placebo-controlled experiments.

Experiment 1: Randomness in Response Generation. Twenty-seven participants performed a random number generation (RNG) task before and during 20 minutes of real or sham tSMS over the left DLPFC in a crossover design. The randomness index, based on entropy and correlation measures, significantly increased under real stimulation ($p < 0.01$), suggesting an inhibitory effect on habitual response patterns.

Experiment 2: Creativity and Physiological Measures. Forty-one participants completed creativity tasks (Alternative Uses Task and CREA test) before and after 30 minutes of real or sham tSMS over left DLPFC, with simultaneous electroencephalography (EEG) and electrocardiography (ECG) recordings. No significant differences were found between conditions in creativity scores or heart rate variability parameters.

Experiment 3: Coordination Games and Risk-Taking Measures. Thirty-six participants (18 dyads) simultaneously engaged in cooperative decision-making tasks that required coordination and strategic interaction, followed by a risk-taking task, after 30 minutes of real or sham tSMS over the left DLPFC. The real stimulation group exhibited significantly higher coordination rates and increased risk-taking behavior ($p < 0.05$).

In conclusion, tSMS over the left DLPFC transiently influenced executive function, enhancing randomness in response generation and strategic risk-taking but it did not affect creativity or autonomic activity. These findings suggest a role for tSMS in cognitive modulation, highlighting its potential applications in neuroscience and in the treatment of neuropsychiatric disorders related to inhibitory control and decision-making.

Effortful access to food enhances the learning of a value-based task in rats

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Finding nutrients regularly is critical to survival, so animals generally invest a great deal of effort in securing access to food. Succeeding in these activities depends on the animal's ability to learn about the environment and the consequences of its behavior. In modern societies, food is highly accessible and the motivation to engage in a 'smart' interaction with the environment, which shapes our reward system, is lower. To test whether effortful access to food has some cognitive benefit, a group of Long-Evans rats were housed with a food dispenser specifically designed to reduce accessibility to food (Low Accessibility, LA). Another group had free access to food (High Accessibility, HA). Both groups gained weight exponentially with no statistically significant differences between them. We then compared LA and HA in a value-based task, in which rats had to choose between two options, one of which was rewarded with water 80% of times (optimal response) and the other only 20% (non-optimal response). They had to learn by trial and error which was the optimal response and, after choosing it on 6 consecutive trials, the reward probabilities were reversed. We found that the LA group learned to solve the task faster than the HA. Specifically, the number of completed trials was significantly higher in LA compared with HA after the first week. Interestingly, the performance of both groups reached the same level later, suggesting an important effect of food accessibility on learning rather than overall decision-making performance. Future experiments will explore the neural correlates of this behavioral effect in the ventral tegmental area and the prefrontal cortex, key areas in learning and decision-making. These results may help to understand poor decision-making and to develop better therapeutic strategies for eating disorders and other unhealthy habits.

Enhancing stress resilience: The protective role of environmental enrichment

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Stress resilience is crucial to protect against neuropsychiatric disorders, such as depression and anxiety, and it can be improved through a variety of strategies, although the exact mechanisms are not yet known. In the case of rat models, social isolation has been shown to act as a high stressor, impairing brain function and increasing vulnerability. In this study, we analyzed the effect of environmental enrichment (EE) as a preventive strategy against social isolation. For this purpose, thirty-two rats were randomly distributed into four groups (n= 8/group): 1) basal (no stimuli); 2) behavioral control (BehC; behavioral testing); 3) social isolation (SI; 4 weeks of cage isolation followed by behavioral testing); 4) prevention (Prev; 4 weeks of EE followed by social isolation and behavioral testing). Behavioral assessments included a sucrose preference test (SPT), to evaluate anhedonia, and elevated zero maze (EZM) to evaluate unconditioned anxiety. Once the behavioral evaluation finished, rat brains were extracted and processed for cytochrome C oxidase (CCO) activity determination, a marker of metabolic activity. CCO was measured in key brain regions: prefrontal cortex, dorsal and ventral hippocampus, nucleus accumbens, and ventral tegmental area. Results showed that SI group exhibited increased anhedonia and anxiety levels, and elevated CCO activity in all brain regions studied. In contrast, the Prev group displayed reduced anhedonia and anxiety compared to SI group, and their CCO activity levels were similar to the basal group, except for a slight elevation in the ventral hippocampus. These findings suggest that prior EE is a protective factor against the adverse effects of social isolation, promoting resilience at both functional and metabolic levels. Increasing knowledge about the effects of protective strategies on the brain, like EE, will facilitate the design of interventions for vulnerable individuals to improve their resilience to stress.

Epileptiform activity induces sex-dependent behavioral changes

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Epileptiform activity (EA) refers to abnormal electrical discharges in the brain, typically detected by electroencephalogram. While commonly associated with epilepsy, EA is also observed in aging and neurological disorders such as autism spectrum disorder and Alzheimer's disease. Although it does not always lead to seizures, EA can contribute to cognitive and emotional deficits.
Despite its clinical relevance, the precise impact of EA on cognition remains poorly understood, largely due to the absence of standardized and well-defined experimental models. In many cases, EA occurs alongside other pathological features of neurological disorders, making it difficult to isolate its direct effects from those of the underlying condition. To address this, we use an intra-hippocampal kainic acid (KA) injection model, which induces EA without triggering seizures. Behavioral assessments were performed 50 days post-induction to evaluate changes in emotional and cognitive functions.
Our findings reveal sex-dependent effects: females exhibit cognitive impairment following EA induction, whereas KA-treated males show no significant cognitive alterations but exhibit increased locomotor activity. To investigate whether these behavioral changes correspond to modifications in brain activity, we are performing in vivo electrophysiological recordings in the hippocampus of freely moving mice. Preliminary data suggest that EA alters high-frequency oscillations in female mice.

Evaluation of the long-term cognitive and neurochemical effects of psilocybin in a chronic stress mouse model

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Depression is a common and incapacitating mental illness. Despite its multifactorial nature, chronic stress is considered a major risk factor for its development. Depression is essentially an affective disorder; however, it can also affect other symptomatic domains, such as cognition. The psychedelic alkaloid psilocybin has been posited as a fast-acting antidepressant, with potential benefits on the aforementioned cognitive symptomatology.
We aimed to evaluate long-term cognitive and neurochemical effects of psilocybin in a chronic multimodal stress model (CMMS).
Adult C57BL/6J male and female mice were assigned to vehicle, psilocybin, CMMS, or CMMS+psilocybin groups. CMMS mice underwent social isolation, restraint and alternating night stressors for 14 days. Two doses of saline/psilocybin (1mg/kg, i.p.) were administered on days 7 and 14. Adrenal gland weight, bodyweight and food-intake were evaluated along with Barnes Maze test (BM) and cortical monoamine concentration. All results were analysed using three-way ANOVA.
CMMS mice showed decreased bodyweight, and increased food-intake and adrenal gland weight. In BM, all mice exhibited task learning. No effects of stress or psilocybin were found in acquisition or reversal learning phases in males. Conversely, female stressed subjects demonstrated an enhancement in test performance during acquisition but not reversal phase, with no effect of psilocybin. Tissue monoamine evaluation revealed an increase in cortical noradrenaline and dopamine concentrations in stressed groups.
In conclusion, the stress protocol triggered physiological impairments, revealing an increased activity of the hypothalamic-pituitary-adrenal axis. The applied stress intensity did not induce adaptation failure and instead promoted successful, sex-dependent behavioural adaptations. Psilocybin was not able to modify previously non-impaired behaviours. Alternative stress protocols exhibiting cognitive dysfunction should be considered for future studies.

Supported by MCIN/AEI/10.13039/501100011033 (PID2021-123508OB-I00) and the Basque Government´s Department of Education (IT-1512–22). E.H.-H: FJC2022-048338-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGeneration EU/PRTR. N.M-A: predoctoral fellowship from Basque Government (PRE_2022_1_0256).

Exploring the impact of a brief and intense exercise protocol on cognitive and psychophysiological variables in a sample of young people with problematic alcohol use

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Physical activity may serve as an effective adjunctive intervention for individuals with risky substance use to improve psychological and cognitive variables. Our study investigated the impact of a brief and intense exercise protocol on cognitive and psychophysiological variables in a young sample with risky alcohol use (RAU) and a control group (CO).
Participants exceeding 150 minutes of moderate/vigorous activity per week were excluded. Volunteers (RAU=44, CO=50) were categorized based on their AUDIT scores (RAU: ♂≥8, ♀≥6). The intervention involved 12 minutes of cycling (RAU=26, CO=30) or stretching (RAU=18, CO=20). Before and after the session, we conducted a resting EEG, and participants evaluated their craving, state anxiety, and perceived effort (Borg Scale). They also completed neuropsychological tests on attention, processing speed, and executive functions (d2; TESEN 3 and 4; Letters and Numbers; 5-digits). Additionally, we analyzed lactate levels, recorded heart rate variability (HRV), and collected saliva samples at various time points (before and 10 minutes after the exercise).
Cycling session improved phonological fluency, reduced execution time on the TESEN 4 for the RAU group and decreased the theta/beta ratio. Regardless of the type of exercise, the RAU group improved their performance on the Letters and Numbers test. Additionally, both groups experienced an increase in lactate levels and heart rate after intense exercise. The RAU-stretching group exhibited a trend towards higher RMSSD, but there were no significant differences in other HRV-related metrics. While objective measures of cardiac performance were similar, the Borg Scale indicated that the RAU group perceived greater effort after the cycling session. Cortisol levels increased in both cycling groups post-exercise, while the increase in irisin at T3 was independent of group and exercise type.
The results suggested that brief, but intense exercise protocols can induce cognitive and psychophysiological changes that may be beneficial for people with RAU.

Exploring the Therapeutic Role of the CB2 Receptor and the Serotonergic System in a Murine Model of Dravet Syndrome

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Dravet Syndrome (DS) is a rare childhood epilepsy caused by SCN1A gene mutations, leading to intense seizures and cognitive impairments through abnormal neuronal hyperexcitability via Nav1.1 sodium channels.

Seizures in DS patients resist conventional treatment, leading to cognitive impairment, making alternative approaches essential for effective management. We focused on fenfluramine (FFA), a serotonin releasing agent, and Ro6866945, a CB2 receptor agonist developed by Hoffmann La Roche.

This study aimed to evaluate whether the co-administration of FFA and Ro6866945 enhances their individual effects. It examined treatment impact on survival and behavioural alterations in DS animals. The study used conditional knock-in mutant mice created using Cre-loxP technology (Syn1-Cre/Scn1aWT/A1783V), to which Ro6866945 (30 mg/kg) and FFA (7.5 mg/kg) were administered individually or in combination, every two days starting from postnatal day 10 (PND10) until postnatal day 24 (PND24). Throughout this period, both motor reflex and weight progression were recorded on alternate days, and on PND23 and PND24 behavioural tests were conducted to evaluate the treatment effect on motor deficits, cognitive impairment, and autistic traits observed in DS mice.

The results reveal that all the survival rate, the motor reflex progression in hind legs, hyperlocomotion test, plus-maze and social interaction test were improved with all treatments in this mice model. These findings suggest that both FFA and Ro6866945 treatment may have benefits beyond seizure reduction and may be beneficial for some aspects of neurological or motor functioning, but the combination did not show better results than the administration of both drugs individually. Additional studies could further explore the mechanisms behind these effects and determine how they can be applied effectively in the DS.

Impact of adolescent stress on behaviour and neuroimmune function

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The understanding of stress-related psychiatric disorders is needed to explain the alarming high rise of these disorders in adolescence. Indeed, adolescent individuals are more sensitive to certain stressors, emphasizing the importance of adolescence as a vulnerable period for the onset of stress-related psychiatric disorders. Chronic stress has been linked with neuroinflammation, which is thought to be partly mediated by specific alterations in glial cells. Nevertheless, the mechanisms behind this disruption remain unclear, since glial cells have long been disregarded. In this sense, we hypothesized that disruptions in the kynurenine pathway in glial cells, which metabolizes the essential amino acid tryptophan and is upregulated by inflammatory signals, could explain the impact of adolescent chronic stress on behaviour.

To mimic both the psychological and biological components of stress during adolescence, we used a double hit model consisting of a chronic stress protocol (Postnatal day (PD)30 - PD58) in male and female mice, which combines social isolation with the oral administration of corticosterone. Following this protocol, a subset of behavioural tasks were performed to assess anxiety-like behaviour (Elevated Plus Maze), sociability (V-SOC task and direct social interaction in an Open field) and depressive-like behaviour (Sucrose Splash Test and Forced Swimming Test). Chronic stressed mice presented behavioural alterations such as depressive-like behaviour and anhedonia. Moreover, after the behavioural evaluation, we extracted different brain regions to analyse potential changes related to neuroinflammation and the kynurenine pathway.

Altogether, this study provides novel behavioural and neuroimmune insights linking adolescent stress with specific behavioural alterations. It remains to be investigated the downstream effect of this metabolic disruption and the potential use of therapies that tackle this pathway.

Impact of esketamine treatment on the lipid profile and mitochondrial activity of PBMC and platelets in patients with resistant major depression

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Treatment-resistant depression (TRD) is characterized by a low response rate to conventional treatments, with persistent symptoms that significantly affect health and quality of life. Esketamine nasal spray has emerged as a rapid-acting antidepressant with a well-established safety and efficacy profile. Its antidepressant effects have been linked to various mechanisms of action, including its influence on mitochondrial energy metabolism.
To investigate the impact of esketamine on mitochondrial activity and the lipidomic profile in patients with TRD, we developed microarrays using peripheral blood mononuclear cells (PBMCs) obtained from blood samples of 12 TRD patients and 6 healthy volunteers. Mitochondrial activity was assessed by measuring the activity of key mitochondrial enzymes, including NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c oxidase (complex IV), glycerol-3-phosphate dehydrogenase (a mitochondrial shuttle enzyme), and monoamine oxidase (MAO) before and after esketamine treatment. Additionally, the activity of complex IV was determined spectrophotometrically in platelets. Lipid profiling was performed using matrix-assisted laser desorption/ionization (MALDI) analysis to evaluate changes in the lipid composition associated with esketamine treatment.
The results showed that intranasal esketamine treatment significantly reduced depressive symptoms, as demonstrated by scores obtained on the MADRS and BDI II scales. Moreover, the activity of complex IV, in PBMCs and platelets, was significantly reduced in TRD patients compared to healthy volunteers. However, following esketamine administrations, the activity of this complex increased significantly in both cell types. Moreover, lipid profiling revealed a significant correlation between certain lipid species and MADRS score.
Thus, even though further research is needed, initial findings suggest that the identified blood cell biomarkers could be useful for monitoring the efficacy of esketamine treatment in patients with DRT.

Impact of neonatal immune activation on adult behaviour and gut microbiome in rats

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In recent years, the gut-brain axis has gained relevance because of the interaction between the nervous and digestive systems, although the underlying biological mechanisms remain unknown. Furthermore, early-life adversities can induce long-term changes ultimately affecting adult behaviour.
This study investigated the effects of early acute immune activation on adult behaviour during the critical phase of neurological development. On postnatal day 14, a crucial stage for neurodevelopment and behaviour, male and female Wistar rats were intraperitoneally administered Escherichia coli lipopolysaccharide (LPS) or saline solution as controls. During the juvenile stage, the animals underwent behavioural assessments, including the forced swim, elevated zero maze, prepulse inhibition, and Morris water maze tests. Brain tissue was collected for histochemical processing and cecum samples were obtained for microbiome genetic analysis.
The behavioural results revealed effects of LPS treatment and sex-related differences. In the elevated zero-maze test, a significant effect of sex was found, with males showing higher anxiety levels. In the forced swim test, LPS-treated subjects showed greater immobility, particularly in female. In the Morris water maze, significant treatment-related differences were found in the spatial memory probe on the final day, with LPS-treated males spending less time in the correct quadrant than controls. In the prepulse inhibition (PPI) test, LPS-treated animals showed altered PPI. Moreover, microbiome sequencing data revealed significant differences in the populations of various species between LPS-treated subjects and their respective control groups.
In addition, significant correlations were detected between the relative abundance of several bacterial species and families and behavioural variables, suggesting that acute neonatal immune activation has long-term effects on the microbiome and could influence behaviour in response to acute stress. Further studies are necessary to understand the relationship between microbiome variations and behavioural changes.
This research was funded by the research project PID2022-140980NB-I00, granted by Spanish Ministry of Science and Innovation.

Individual differences in the interplay between predictive processing and executive control among subclinical traits

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Prefrontal cortex-related executive functions and probabilistic sequence learning, which underlie predictive processes, interact in both cooperative and competitive ways. Probabilistic sequence learning involves acquiring regularities from the environment without conscious awareness, while inhibitory control enables the suppression of irrelevant information during a task. However, individual differences in the aforementioned complex and dynamic interaction remain largely unexplored. We will explore how the co-occurrence of traits, frequently observed in subclinical populations, influences the dynamic relationship between probabilistic sequence learning and inhibitory control. To address this gap, our study aimed to investigate how traits associated with Attention Deficit Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), and Obsessive-Compulsive Disorder (OCD) influence this dynamic relationship. These neurodevelopmental traits share common features across neurobiological and behavioral levels, and these features also manifest at a subclinical level in the general population. Applying a spectrum-based approach, we recruited a healthy, non-clinical sample via an online experiment. Participants completed questionnaires assessing ADHD, ASD, and OCD traits. To measure the interaction between executive functions and probabilistic sequence learning, we developed a novel task that integrates probabilistic sequence learning and response inhibition within a single paradigm. Our results demonstrated significant probabilistic sequence learning effects, as indicated by both reaction time and accuracy measures. Participants with higher OCD scores demonstrated enhanced probabilistic sequence learning performance. Conversely, individuals with elevated ADHD scores exhibited diminished inhibitory control. Furthermore, individual differences across the three traits influenced inhibitory control. These results underscore the intricate relationship between subclinical traits and cognitive functions, showing that while probabilistic sequence learning remains intact, individual differences in OCD, ADHD, and ASD significantly influence inhibitory control.

Innate Immune Regulation of Memory: TLR9's function in the Hippocampus

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Toll-like receptors (TLRs) are innate immune sensors that, beyond their well-established role in inflammation, also influence cognitive and emotional regulation under physiological conditions. However, the precise mechanisms underlying these effects remain unclear.

Given the crucial role that synaptic plasticity and metabolic homeostasis play in cognitive processes, this study explores the specific impact of TLR9 on memory in mice. We assessed episodic memory using the novel object recognition test and working memory with the Y-maze, while simultaneously examining the underlying molecular mechanisms in the hippocampus.

Our results show that the activation of TLR9 significatively modulates both episodic and working memory. Interestingly, TLRs can signal through different adaptor proteins, and TLR9-mediated cognitive mechanisms may be mediated by SARM1, an adaptor predominantly expressed in neurons, suggesting a neuron-specific TLR9 signaling. Additionally, our findings indicatethat the mammalian target of rapamycin (mTOR) pathway may participate in the interaction between TLR9 and neuronal function, as evidenced by changes in the expression of proteins that activate mTOR and markers of synaptic plasticity..

In conclusion, these findings suggest that TLR9 plays a key role in immune-metabolic regulation, thereby influencing neuroplasticity, cognition, and behavior.

Input-Output circuit mapping to the Higher-Order Relay Laterodorsal Nucleus of the Thalamus in Mice

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The laterodorsal nucleus (LD) of the rodent has traditionally been associated with the anterior nuclear group of the thalamus (ATN) and hence with the mnemonic and emotional functions of the "limbic system". In recent years, however, electrophysiological studies have shown that LD neurons process mainly visuo-spatial and visuo-motor signals. Moreover, available anatomical data on LD connections suggest that may be part of the complex multi-regional forebrain networks that create and update the representation of the surrounding space. These representations critically underpin navigation, and scene recall. The input-output circuit motifs of ATN has not been mapped with high-resolution, micropopulation and single-cells methods.
On adult wildtype C57BL/6 mice, we are analyzing the neurochemical architecture of LD and carried out a brain-wide mapping of its input output connections using anterograde and retrograde labeling methods. Specifically, we mapped and quantified the sources of cortical and subcortical input to different LD subregions and mapped and compared the distribution and terminal structure of their axons. Results to date indicate that LD receives robust input from several subcortical structures involved in visuomotor control such as the pretectal nuclei, the superior colliculus and the pregeniculate nucleus. In addition, LD receives convergent cortical afferents from retrosplenial, posterior parietal and primary and secondary medial visual areas as well as from the extended hippocampal system. These projections include both layer 5 and layer 6 corticothalamic inputs. In turn, LD neurons innervate the cingulate, retrosplenial, visual and parahipocampal areas, in complex patterns multiarea patters, mainly through bifurcated or trifurcated axons.
Along with the available functional data, our anatomical findings support the notion LD neurons may compute signals related to eye motion and visual landmarks and selectively feed the resulting signals to specific cell groups of neocortical and parahippocampal areas involved in mapping of surrounding space.

Integrative Study of Operant Behavior and In Vivo Electrophysiology: Long-term Impacts of Adolescent Tetrahydrocannabinol Exposure on Addiction Vulnerability in Mice

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Substance abuse during adolescence is one of the variables known to increase an individual's risk of suffering from addiction later in life. The lack of maturation of brain areas involved in impulse control increases both the probability of engaging in risky behaviors, such as drug abuse and the susceptibility of the adolescent brain to negative, long-term consequences of drug exposure.

In this context, cannabis is one of the most common illegal drugs in Europe, and the prevalence of cannabis use among adolescents is exceptionally high. Tetrahydrocannabinol (THC), the main psychoactive component of cannabis, elicits most of its effects on the brain through cannabinoid receptor 1 (CB1). This receptor is widely expressed throughout the brain, especially in the orbitofrontal cortex (OFC) and the hippocampus (HPC).

Remarkably, both the OFC and the HPC are often altered in cannabis-abusing individuals, and they are involved in the anticipation stage of the addiction process, as well as the regulation of emotionally based decision-making. Thus, we hypothesize that exposure to THC during adolescence would impact the maturation of these brain areas, leading to an increase in vulnerability to addiction.

We used a mouse model of food addiction, based on operant behavior maintained by palatable food, to study how early, chronic exposure to THC can influence mice´s vulnerability to develop addictive-like behavior. We combined this approach with electrophysiology in vivo techniques, so that we could study potential alterations in the neural activity and connectivity of the HPC and OFC that can be correlated with addiction and/or THC exposure. We also studied other behavioral domains that are relevant in the context of cannabis abuse, such as anxiety, memory, sociability, and psychosis. So far, we have obtained relevant results in the study of compulsivity and working memory, which pave the way for further research on this topic

Involvement of Specific brain Pathways in Cannabis Use Disorder

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Addiction is a complex and chronic disorder characterized by compulsive drug-seeking behavior, the emergence of a negative emotional state upon withdrawal, and a loss of control over intake despite repeated failed attempts to abstain. Cannabis Use Disorder (CUD) has gained increasing relevance, with global cannabis consumption rising from 5.7% in 2015 to 7.7% in 2022 among adults aged 15-64 in Europe. Additionally, according to the UNODC World Drug Report (2023), cannabis remains the most widely used illicit substance, reflecting the urgent need to understand its neurobiological underpinnings.

Despite its rising incidence, the lack of valid animal models has hindered the study of its underlying neurobiological mechanisms. In this study, we combined a mouse model of CUD using WIN 55,212-2 self-administration, incorporating DSM-5 criteria and phenotypic vulnerability traits with a chemogenetic approach to inhibit specific brain pathways. The study specifically explored the role of glutamatergic pathways, including projections from the basolateral amygdala (BLA) and hippocampus (HPC) to the nucleus accumbens core (NAcC), which are critical for addiction-related behaviors. To evaluate the impact of these circuits, we employed chemogenetic inhibition using a Retro-DREADD approach. Significant reductions in neuronal excitability of these pathways, via hM4Di activation by the designer drug Deschloroclozapine (DCZ), affected cannabis-seeking behavior and compulsivity. This study sheds light on new neurobiological mechanisms underlying vulnerability to CUD, which could pave the way toward novel and efficient interventions for this disorder.

Long-Lasting Effects of Early Postnatal Particulate Matter Exposure on Cognition and Behavior in APOE Transgenic Mice

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Environmental pollution is a significant public health concern, with extensive evidence linking air contaminants to severe health effects. Among air pollutants, particulate matter (PM) is particularly worrisome due to its capacity to reach the central nervous system, triggering oxidative stress and neuroinflammation. Early postnatal exposure to fine PM can disrupt neurodevelopmental processes, altering brain structure and function, and increasing susceptibility to neurodegenerative diseases later in life. The apolipoprotein E (APOE) gene is polymorphic in humans, with APOE3 being the most prevalent allele in the population. In contrast, APOE4 is the strongest genetic risk factor for Alzheimer's disease, particularly in females, and has been associated with differences in blood-brain barrier integrity, synaptic plasticity, and neuroinflammation. Moreover, APOE3 and APOE4 differ in their susceptibility to environmental toxicants, with APOE4 carriers exhibiting increased vulnerability. In this sense, this study aimed to determine the effects of early postnatal exposure to inhaled PM10 (SRM 2787; NIST) from postnatal day 4 to day 14 in whole-body exposure chambers on aging-related processes in ApoE3 and ApoE4 humanized transgenic mice. At 9 months of age, the mice performed the Open Field test to assess activity and anxiety-related behavior and the Morris Water Maze (MWM) test to evaluate spatial learning and memory. Following behavioral assessments, mice were scarified and brains collected for biochemical analysis. Our results revealed genotype-specific behavioral differences, with PM exposure differentially affecting each. Unexpectedly, ApoE3 mice showed signs of hyperactivity, particularly in females, and a bad performance of MWM test. Additionally, PM-treated mice displayed an anxiolytic-like behavior, suggesting altered sensitivity to novel environments. In contrast, PM-exposed APOE4 mice exhibited impaired short-term memory, with significant sex differences in long-term memory consolidation. These findings highlight the complex interplay between genotype, sex, and environmental factors in neurodegeneration. Ongoing biochemical analyses will provide further insight into the underlying mechanisms.

Mind wandering simultaneously disrupts inhibition and enhances statistical learning

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Mind wandering, the ubiquitous shift of attention away from the present task, has been predominantly viewed as a cognitive liability, disrupting executive functions like inhibitory control. Yet, emerging evidence points to its potential benefits, particularly in enhancing statistical learning – the implicit acquisition of environmental regularities. This study aimed to elucidate the complex interplay between mind wandering, inhibitory control, and statistical learning using a novel task (Cat-No-Go task) that integrates both measures within a visuomotor paradigm. We recruited healthy young participants for an online study involving a visuomotor statistical learning task. This task, crucially, incorporated specific target stimuli that required participants to exercise inhibitory control, allowing us to assess both learning and inhibition concurrently. Our findings demonstrate a clear dissociation: increased mind wandering was associated with impaired inhibitory control, as evidenced by reduced accuracy in suppressing responses to specific targets, but simultaneously facilitated probabilistic learning, reflected in improved pattern detection. We propose that this dual effect might be mediated by frontal slow waves, which could disrupt executive networks while concurrently enhancing neural processes involved in consolidating learned statistical regularities. These findings challenge the purely detrimental view of mind wandering and underscore its complex, multifaceted role in cognition.

Modulation of neuronal activity and learning in rats: effects of prelimbic cortex silencing in individual and cooperative operant conditioning

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The acquisition of new behaviors involves the interaction of multiple brain regions and the reorganization of their respective neural activities. In this study, we analyzed the dynamics of neural activities in rats during a temporal-component operant conditioning task, in which the animals had to remain on a platform for at least one second to receive a reward. Local field potentials (LFPs) were recorded in the prelimbic cortex (PrL), the basolateral amygdala, the nucleus accumbens, and the CA1 region of the hippocampus.

Collected results indicated that, during the initial phases of learning, unconditioned rats exhibited higher beta-band activity, associated with attention and active processing. As the behavior became consolidated, low-gamma activity increased, reflecting greater neural efficiency and task automatization. To assess the role of the PrL in this process, we performed a reversible silencing of synaptic output in this region using virus-delivered silencing of synaptic transmission (next generation vINSIST-2 system). Rats with blocked PrL output exhibited a significant impairment in performance, reflected in a lower number of rewards obtained. Additionally, electrophysiological recordings showed alterations in neuronal activation patterns in related regions, indicating that the PrL is crucial for goal-directed behavior modulation and the coordination of responses in tasks with temporal demands.

Furthermore, we extended our analysis to a cooperative version of the task, where pairs of rats had to climb the platform together to obtain the food pellet. This experiment provides insights into the neural mechanisms underlying cooperative learning and decision-making, shedding light on how social dynamics influence neural activity and behavioral adaptation.

Our findings highlight the importance of the prelimbic cortex in learning control and neural efficiency, providing key insights into the reorganization of brain circuits during both individual and cooperative behavior acquisition.

Financial support was kindly provided by Ministerio de Ciencia e Innovación to JMDG and AG (BIO122&PID2021-122446NB-I00).

Molecular characterization of the dopaminergic system in the Cntnap2 knockout mouse model of autism

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Alterations in social behaviour are one of the core deficits in Autism Spectrum Disorder (ASD). Although it is unclear what circuits underlie such deficits, numerous authors have proposed the idea that imbalances in the mesocorticolimbic dopaminergic system could be one of the leading causes. In fact, dopaminergic neurons located in the ventral tegmental area (VTA) that innervate the nucleus accumbens (NAcc) and the cortex (CTX) are critical for the creation of reward associations, including social reward.
We have previously identified functional dopaminergic alterations in the Cntnap2 KO mouse model of ASD. In this study, we further characterize these alterations at the molecular level by analyzing the expression of dopamine-related genes, using quantitative PCR (qPCR). On one hand, we examine dopaminergic genes in which mutations and/or genetic variants have been associated with ASD, including those encoding dopaminergic receptors and the dopamine transporter, among others. On the other hand, we analyze the expression of genes involved in dopaminergic transmission, including its synthesis, metabolism and release, as variations in these genes have been linked with interindividual differences in several cognitive domains.
Preliminary results show alterations in several dopaminergic markers. These results complete previous functional results in our laboratory and allow us to better understand the mechanisms leading to the deficits of the dopaminergic system associated to social behavior in ASD and inform the development of targeted therapies.

Mu Rhythm During Observation of Grasping and Motor Actions in Real-Life vs. Screen-Based Contexts

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Mu rhythm (~8–12 Hz) in the somatosensory cortex has long been associated with both performing and observing motor activities. This study aimed to investigate how the medium through which motor actions are viewed—whether physical or on-screen—affects this specific brain rhythm. To explore this, 40 participants were shown identical narrative content presented in two formats: a continuous, unedited film and a live theatrical performance. Using electroencephalography (EEG), we measured Mu rhythm activity in the left (C3) and right (C4) somatosensory regions in response to 24 motor actions depicted in each format (24 actions × 40 participants × 2 conditions = 1,920 trials). We calculated the ERSP and PSD across the entire epoch [-1000 to 2000 ms] for the selected electrodes and compared them between the reality and screen conditions. We also calculated ERD percentage values using the formula ERD(%) = (A-R)/Rx100, where A represents the power within the 8–12 Hz frequency range during the post-onset period of the grasp (0–1000 ms), and R refers to the baseline period (-500 to 0 ms), following established methodologies. To assess statistical significance, we conducted paired t-tests with a threshold of $p < 0.05$. Our findings revealed lower Mu spectral power in the somatosensory cortex following the onset of motor actions in the live performance compared to the on-screen version, with a more pronounced effect in the left hemisphere. The observed sensorimotor Mu event-related desynchronization (Mu-ERD) was stronger during real-world observation than screen-based viewing. These results have potential implications for fields that rely on somatosensory processing, such as online learning, virtual reality, and brain-computer interfaces.

Neurofunctional Evidence of Altered Semantic Inhibition in Obsessive-Compulsive Disorder

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Obsessive-compulsive disorder (OCD) is associated with deficits in inhibitory control, linked to dysfunctions in fronto-striatal and cerebellum-cortical circuits, including the anterior cingulate cortex (ACC), supplementary motor area (SMA), and inferior frontal gyrus (IFG). This functional neuroimage (fMRI) study examines the neural correlates of inhibition in OCD using the Hayling Task, consisting of completing sentences (e.g., It was raining in the street, she opened the…) with the most appropriate word (umbrella), or a totally unrelated word (e.g., stairs), thereby assessing semantic association and semantic inhibition processes, respectively.

Two groups of university students —individuals with OCD (n=19) and healthy controls (n=21)- performed the Hayling task while undergoing fMRI. Imaging data were analyzed to examine the effects of group (OCD, control) and semantic condition (associative vs. inhibitory).

The results showed greater activations in the inhibitory compared to the associative condition in the SMA and the left IFG. Focusing on the inhibitory condition, the controls showed more activation than the OCDs in the ACC, the (left) SMA, and the left cerebellum. Also, in the associative condition, the controls showed again more activation in the anterior cingulate, in a small cluster in (right) SMA, and the left cerebellum.

Our findings confirm that individuals with OCD exhibit altered activation patterns in key brain regions (ACC, SMA, and cerebellum) during semantic inhibition, consistently with previous evidence on fronto-striatal and cerebello-cortical dysfunctions in OCD (Tanaka et al., 2003; Zhang et al., 2023). The reduced activation in the ACC and SMA aligns with models of impaired cognitive flexibility and response suppression in OCD (Moore et al., 2024). Additionally, the cerebellum's involvement in semantic inhibition, further supports recent theories proposing its role in higher-order cognitive functions, including prediction and error monitoring during linguistic and executive tasks (LeBel & D'Mello, 2023).

Neurons in the human brain encode rapidly learned visual information to reshape perception

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Humans can swiftly learn to recognize visual objects after only a few exposures. A striking example of rapid learning is the sudden recognition of a degraded black-and-white image of an object (Mooney image). These images are initially unrecognizable but become easily interpretable after a brief exposure to the original intact version of the image. Integrating recently learned information with existing knowledge necessitates forming enduring neuronal representations to enable future recognition. However, the mechanisms underlying how rapid perceptual changes are reflected in the neuronal dynamics induced by learning within the human brain remain poorly understood. Here, we recorded the spiking activity of neurons in medial occipital and temporal regions of the human brain in patients performing an image recognition task that involved rapid learning of Mooney images. Neurons in the human occipital cortex (OC) and medial temporal lobe (MTL) modulated their firing patterns to encode rapidly learned visual information and reshape perception. Population decoding revealed that occipital neurons resolved the identity of learned images at the cost of additional processing time, with delayed responses observed in MTL neurons. Our findings suggest that OC may not rely on feedback from MTL to support recognition following rapid perceptual learning. Instead, learning-induced dynamics observed in OC may reflect extensive recurrent processing, potentially involving top-down feedback from higher-order cortical areas, before signals reach the MTL. These results highlight the need for further computation beyond bottom-up visual input representations to facilitate recognition after learning and provide spatiotemporal constraints for computational models incorporating such recurrent mechanisms.

Novelty detection and predictive processing impairments in an animal model of Alzheimer's disease

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Aging reduces peripheral processing accuracy, increasing reliance on top-down predictions over bottom-up sensory input, a process further impaired in dementia. In Alzheimer's disease (AD), temporo-parietal dysfunction affects auditory adaptation, exacerbating cognitive and behavioral deficits. To investigate how dementia impacts deviance detection, we used TgF344-AD rats, which exhibit AD-related lesions, including amyloid plaques and hyperphosphorylated tau. We trained 32 Fischer 344 rats (16-month-old TgF344-AD and controls) to discriminate deviant tones in an oddball paradigm.

To assess discrimination ability, we implemented the auditory oddball paradigm with different interstimulus intervals (ISI: 1.5, 2, and 4 s), frequency contrasts (0.5, 1.0, and 1.25 octaves) and deviant probabilities (10 and 30%). Additionally, we used a many-deviant paradigm with several frequency contrasts. Animals activated a nosepoke when detecting a deviant sound, receiving a reward pellet. Performance was evaluated using the d' index.

Following behavioral experiments, neuronal spiking activity was recorded in the auditory cortex using the same auditory stimulation paradigms. Stimuli were 75 ms pure tones at behaviorally tested frequencies, presented at a rate of 4 stimuli/s.

Results show that TgF344-AD rats required more sessions than controls to learn the nosepoke-reward association. They also needed more sessions to achieve a d'>1 in the training task and maintained lower d' scores. Control animals outperformed TgF344-AD rats across all paradigms, with both groups performing best at a 4 s ISI. There were sex differences in the performance and all groups performed better when deviant probability was 10%. In the many-deviant paradigm, control rats showed better performance with higher frequencies, while TgF344-AD rats performed better at lower frequencies. These findings suggest that AD-related pathology disrupts auditory deviance detection, potentially mirroring impairments observed in human patients.

Work suppported by AEI (grant #PID2023-148541OB-I00) to MSM/DPG; The Foundation Ramón Areces grant CIVP20A6616 to MSM/LQV/DPG, and JCYL (SA218P23) to MSM.

Phoneme encoding in the inferior colliculus of gerbils with and without noise-induced cochlear synaptopathy

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Numerous studies have explored how auditory information processing at the peripheral level is altered following synaptopathy, but primarily using basic acoustic stimuli (e.g., transients, noise, tones). However, speech stimuli are rarely considered, despite their importance in linking speech intelligibility deficits to synaptopathy. In this study, we utilized filtered real human speech phonemes designed to distinguish between temporal fine structure (TFS) and temporal envelope (TENV) encoding (see Gaudrain & Baskent). These were presented as stimuli to record neuronal responses in the inferior colliculus of gerbils—both control animals and those with noise-induced cochlear synaptopathy (CoSy). CoSy was induced in awake, freely moving gerbils, that were exposed to an octave band of noise (2.6–5.2 kHz) at 100 dB SPL for two hours in an antiparallel chamber, where animals were unrestrained within individual compartments of a subdivided cage (one gerbil per cell). CoSy was assessed using multiple non-invasive measures, aligned with human studies, including auditory brainstem responses (ABR), distortion product otoacoustic emissions (DPOE), and cortical auditory evoked potentials (CAEP), with validation through immunohistochemistry to quantify sensory cell and synapse counts in the cochlea.
Here, we present findings categorizing neuronal responses in the inferior colliculus of anesthetized gerbils to various speech stimuli and relate these responses to each neuron's frequency response area. Additionally, we examines how different masking conditions influence these responses and assessed the effectiveness of neuronal coding in representing the acoustic stimuli. Lastly, we evaluate how noise-induced cochlear synaptopathy impacts these coding mechanisms and their efficacy.
We found that CoSy induces profound, yet subtle, changes encoding that could easily be missed by traditional electrophysiological assays. Further, increased spontaneous rates and thus increased side-band inhibition that reduces neuronal firing is likely compounding coding impediments in noisy situations.

Work supported by ERA-NET NEURON JTC 2020 (CoSySpeech Project) and Junta de Castilla y León (grant SA218P23).

Response of Emx1 and Otp glutamatergic neurons of the amygdala to odorants with different biological relevance

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Previous studies reported the importance of olfactory signals on rodent behavior, highlighting the role of the amygdala as a valence-encoding structure. In rodents, exposure to a synthetic component of red fox secretions (TMT) leads to freezing behavior and expression of cFos in different subdivisions of the amygdala. However, all amygdala subdivisions are composed of a wide variety of cell types with different embryonic origins, which conditions much of their phenotype and function. Here, we study the expression of cFos to odorants with different biological relevance in two glutamatergic neuron subpopulations of the amygdala with different embryonic origins (Emx1 derived from the pallium and Otp derived from the telencephalon-opto-hypothalamic domain). To label those cells, we used a transgenic mice strain obtained from crossing Emx1-Cre; Rpl22HA with Otp-eGFP, in which Rpl22HA is an Emx1-specific reporter and eGFP is an Otp-specific reporter. We performed behavioral tests with these mice, exposing them to neutral (H2O), frugal (pentyl acetate) or predator (TMT) odors, and studied the cFos activation of Emx1 versus Otp neurons in different amygdala subdivisions by way of triple immunofluorescence. We found abundant cFos+ cells in several olfacto-recipient cortical amygdalar areas, most of which were Emx1 cells, without apparent differences between odorants. We also found cFos cells in all subdivisions of Me, some of which were Otp cells, while others were Emx1 or remained unidentified (possibly GABAergic), with variable results in different subdivisions. Our results can help to understand the involvement of different subpopulations of glutamatergic neurons of the amygdala in odor-mediated behavior.

Funding: Spanish Ministerio de Ciencia, Innovación y Universidades / Agencia Estatal de Investigación (MICIU/AEI, PID2023-151927OB-I00), and Generalitat de Catalunya / AGAUR (2021 SGR 01359). JF has a predoctoral contract from the Spanish Ministerio de Ciencia, Innovación y Universidades (FPU22/03133).

Role of clustered protocadherins in engram formation and memory function

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The brain encodes learned information as specific changes to engram cells, populations of neurons that act as building-blocks of memory. These cells, whose activity is sufficient and necessary for the recall of the memory, modify their connections across brain regions in a way that sustains the physical storage of information. Given the relevance of connections between engram cells, we investigate whether transsynaptic adhesion molecules regulate engram function. In particular, we assess the role of clustered protocadherins (cPcdhs), a superfamily of cell surface proteins that act as a barcode system for synapse specification. Using engram labelling technology, in situ hybridization, spatial transcriptomic and genetic manipulations, we examine the impact of experience-encoding in cPcdhs expression in the hippocampus. We further investigate the role of cPchds in engram biology. Our findings suggest that cPcdhs play a significant role in shaping memory formation and function, influencing the dynamics of engram cells.

Selective mu antagonist CTOP blocked spatial preferences induced by electrical stimulation of the Parabrachial Complex in female rats

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Controversy persists in current research about the involvement of dopaminergic and non-dopaminergic circuits in the brain reward system and their differential role in the processing of some of its specific components. In this regard, some of our previous studies have identified a small brain structure in the first central levels of the visceral-vagal information relay, the external lateral parabrachial subnucleus (LPBe), whose activation induces preference for associated gustatory and spatial stimuli. In the same line, other researchers recently found that the vagal-brain axis is involved in processing rewarding signals from the gastrointestinal tract.

Mu and kappa opioid receptors are present in this parabrachial region, and the aim of the present study is to elucidate the differential involvement of these receptors by using ctop [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2], a selective mu receptor antagonist, to pre-treat a group of female rats subjected to electrical stimulation of the LPBe.
Preliminary results demonstrate that electrical stimulation of the LPBe induces preferences for associated stimuli in a concurrent Conditioned Place Preference [cCPP] procedure, and that these preferences are completely blocked after the administration of the selective mu receptor antagonist ctop. Given the role of the vagal-cerebral axis in modulating reward, these results will be discussed in relation to its involvement in the modulation of the hedonic/affective component and its usefulness for the treatment of affective disorders. (Supported by funds from Plan Propio UGR-FEDER [C-SEJ-348-UGR23]).

Sex- and Gestational-Time-Dependent Alterations in Excitation/Inhibition Balance and Schizophrenia-Like Behaviors Following Maternal Immune Activation

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Schizophrenia (SCZ) is a neurodevelopmental psychiatric disorder affecting approximately 1% of the global population. Its etiology involves complex interactions among genetic, epigenetic, and environmental factors, with notable sex-based differences. Impaired GABAergic maturation is a key contributor to the cognitive and negative symptoms of SCZ, which are poorly addressed by current antipsychotic treatments. Maternal immune activation (MIA) disrupts fetal neurodevelopment and increases SCZ risk. In animal models, MIA induces SCZ-like phenotypes, with behavioral outcomes depending on the gestational timing of exposure. MIA particularly affects GABAergic development in the prefrontal cortex (PFC) and dorsal hippocampus (dHPC). Recent studies indicate that mild MIA at early and late gestational periods induces distinct SCZ-like alterations, most of which are transient, emerging in adolescence and spontaneously resolving in adulthood. However, the neurobiological basis of these differences remains largely unexplored, particularly regarding the excitation/inhibition (E/I) balance in the PFC and dHPC, as well as from a sex-specific perspective.
In this study, we compared positive, negative, and cognitive SCZ-like alterations in adult male and female offspring exposed to mild early or late MIA. Additionally, we evaluated the E/I balance status in PFC and dHPC structures at different developmental stages (adolescence and adulthood) by assessing c-Fos protein expression—a marker of early neuronal activity—in excitatory neurons and inhibitory interneuron subtypes following acute stress.
Our behavioral results reveal sex- and gestational-time-dependent deficits in positive, negative, and cognitive domains associated with SCZ. Ongoing immunofluorescence analyses further indicate sex- and developmentally dependent alterations in E/I balance across PFC and dHPC subdivisions in early and late MIA-exposed mice. Future studies will extend these observations to extracellular matrix components (perineuronal nets, reelin) and glial cells.
Our findings highlight sex- and gestational-time-specific disruptions in excitation/inhibition balance and schizophrenia-like behaviors, providing insights into the neurodevelopmental mechanisms underlying SCZ vulnerability.

SEX DEPENDENT CANNABINOID EFFECTS ON HIPPOCAMPAL FUNCTION

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Tetrahydrocannabinol (THC), the main psychoactive compound in cannabis, has well-documented detrimental effects on cognition, particularly memory impairment. Its amnestic effects are primarily mediated by CB1 receptor interactions in the hippocampus, a key region for memory formation, spatial navigation, and learning. However, recent research suggests that THC's impact on memory differs between sexes, likely due to variations in CB1 receptor expression in different cell types and neural pathways. In this work, we study hippocampal-dependent cognitive processes using a plethora of behavioral tasks aiming at revealing potential sex differences and the participation of neurons and glial cells.

In wild-type male and female mice we used Novel Object Recognition (NOR) and the Barnes Maze to assess non-spatial and spatial memory, respectively. Our results show that THC impairs Barnes Maze performance in males but not females, suggesting sex-dependent pathways for spatial memory with CB1 involvement in males only. In contrast, in the NOR test, THC affects both sexes. However, calcium imaging (fiber photometry) recordings reveal distinct hippocampal activity patterns between sexes during NOR, suggesting different underlying mechanisms despite CB1 involvement in both cases. In parallel, CB1-floxed mice with CB1 deletion from hippocampal principal cells or astrocytes were tested in the cheeseboard maze, an apparatus that allows the study of different types of memory while recording hippocampal cellular activity. Here we observed that neuronal and glial activity behave differently in males and females depending of the cognitive processes measured by the maze. Thus, these results add an extra dimension to the role of hippocampal circuits in the control of memory in males and females. Current experiments are being carried to evaluate the effect of THC on the hippocampal CB1-KO mice in order to elucidate the cell-specific participation of the receptor in cognitive processes.

Sex Differences in Susceptibility to Developing Cannabis Use Disorder

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Cannabis ranks among the most widely used drugs in both the EU and the USA. Defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), Cannabis Use Disorder (CUD) is a substance use disorder characterized by a persistent inability to stop using cannabis, even when it leads to psychological or physical harm. Like other substance use disorders, CUD arises from a complex interplay of environmental factors such as peer influence, stress, and availability of the drug, and genetic factors, including variations in the endocannabinoid and dopaminergic systems and dopamine that influence susceptibility to develop CUD.

Human studies have shown that women are at a higher risk of transitioning to addiction with faster CUD development after their first intake of drugs, and they face more significant challenges in remaining abstinent. Gender dimorphism in CUD may be linked to inherent variations in brain structure, as well as the influence of circulating hormones on motivation, decision-making, reward, and cognitive processes, among other factors. However, research involving humans and rodents has highlighted a substantial lack of data concerning the female cohort. This underscores the urgent need for more research on female data to fully understand the mechanisms underlying the development of addiction in women, keeping the audience motivated and engaged in the field.

Our research is a step towards understanding the neurobiological and behavioral differences in susceptibility to CUD related to sex. By using a mouse model in a drug self-administration protocol with WIN 55,212-2, we found a significantly higher count of addicted individuals among females. Additionally, females demonstrated superior scores in compulsivity and response persistence, both key indicators of addiction. These findings not only highlight the existence of gender dimorphism in CUD but also suggest the potential for new, more effective therapeutic approaches, instilling hope and optimism in the field.

Sex-Dependent Effects of Juvenile and Adult Stress on LPA 1 Receptor Expression and Depression-Like Behaviors in Mice

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Juvenile stress impacts brain development and increases the risk of stress-induced depression, often through inflammation-related mechanisms. Despite known sex differences, research remains scarce. This study examined how stress at different developmental stages affects depression-like behaviors and lysophosphatidic acid receptor 1 (LPA_1 receptor) expression in male and female C57BL/6J mice (N=48, 24 males, 24 females). Given the limited efficacy of antidepressants, alternative mechanisms such as inflammation and the LPA_1 receptor signaling are gaining attention.

Mice were divided into control (C), juvenile stress (JE), adult stress (AE), and combined juvenile-adult stress (DE) groups for each sex. Stress was induced through unpredictable mild stressors. Behavioral assessments included the Open Field Test (OFT) for anxiety-like behavior and locomotor activity and the Tail Suspension Test (TST) for depression-like behavior. Western blot analyses measured the LPA_1 receptor expression in brain tissue.

Results showed that stress increased LPA_1 receptor expression, particularly when stress occurred during the juvenile period, with a stronger effect in females. Pearson correlation analysis revealed a significant positive correlation between LPA_1 receptor expression and immobility time in the TST in males ($r=0.485$, $p<0.05$), suggesting a link between increased LPA_1 receptor levels and depressive-like behavior. No significant correlations were found between LPA_1 receptor expression and behavior in females.

These findings suggest that juvenile stress upregulates LPA_1 receptor, with sex- dependent differences. The correlation with depressive-like behavior in males highlights a potential role for LPA_1 receptor as a biomarker for stress-induced depression, warranting further investigation into its function in sex-specific stress responses.

Funding: PID2020-117464RB-I00, PID2023-151537OB-I00 funded by MICIU/AEI/ 10.13039/501100011033, Consejería de Conocimiento, Investigación y Universidades, Junta de Andalucía (P20_00460), FPU21/05694 and University of Malaga.

Sexual Differences in Stress Response: The Role of Microglia and Neurogenesis

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Stress, especially during sensitive periods of development, can induce neuroplastic changes in brain regions such as the hippocampus, which increases vulnerability to the negative effects of a second stressor during adulthood, precipitating the development of depressive symptoms. Additionally, it is known that depression is more common in females (5.1%) tan in males (3.6%). Consequently, we investigated potential sexual differences in the interaction between microglia and neurogenesis in stress-induced depression. For this reason, male and female C57BL/6J mice were subjected to two stress protocols, the first in the juvenile period and the second in adulthood. Neurogenic, microglial and behavioral changes were analyzed (saccharin preference test and social behavior test). The results revealed that juvenile stress increased saccharin preference in males and decreased this preference in females. In addition, females subjected to stress during both juvenile and adulthood period exhibited anhedonic behavior. Social behavior was affected by the combination of juvenile and adulthood stress in both sexes. At cellular level, adulthood stress reduced neurogenesis in males and impeded neuronal maturation in females. Furthermore, although both juvenile and adulthood stress induced changes in microglial morphology in both sexes, these alterations were differently expressed in males and females. These findings suggest that stress during sensitive periods affects males and females differently, potentially mediated by observed changes in microglia and neurogenesis. In conclusion, juvenile and adulthood stress differentially affect mice based on sex.

This study was supported by PID2020-117463RB-I00 and PID2023-151537OB-I00 funded by MCIN/AEI/ 10.13039/501100011033, to CP and MP-M. PC-P has an FPI grant (PID2020-117463RB-I00).

Sexual differences in stress-responsive behavior and resiliency of adult mice exposed to maternal separation during infancy

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Maternal separation is an early-life adversity that can cause long-term brain and behavioral changes, increasing vulnerability to stress-related disorders later in life. This study aimed to investigate sex-specific responses to adult stress in mice exposed to early adversity. Female and male C57BL/6J mice underwent 3-hour daily maternal separation (MS) for 21 consecutive days. At day 60, they were exposed to a single 2-hour restriction stress (RS) and assessed 24 hours later through a 3-day behavioral test protocol: Nest Building, Open Field and Forced Swimming Tests. The experimental groups included Control, RS, MS, and MS+RS.

Stress responses differed between sexes. RS males performed better in the Nest Building Test, while for females, this behavior was only observed in the MS+RS group. In the Open Field Test, RS males spent less time in the center, while MS females showed increased movement velocity. These variables were unaltered in the MS+RS groups for both sexes. In the Forced Swimming Test, MS+RS females showed increased swimming time but this was not observed in the MS-only group. No significant results were found for males in this test.

Principal component analysis (PCA) revealed that RS males developed maladaptive coping strategies (e.g., faster nest building and increased grooming). MS females were hyperactive in low-threat situations (Open Field) but exhibited passive behavior in high-threat situations (Forced Swimming). However, the MS+RS group exhibited a reduction in maladaptive coping behaviors in both sexes, with females showing increased motivation for nest building and prolonged swimming. In males, the MS+RS combination diminished stereotypic behaviors.

In conclusion, these sex-specific behavioral responses to stress provide insights into the neurobiological basis of susceptibility or resilience to disorders such as depression.

Funding: PID2020-117463RB-I00, PID2023-151537OB-I00 funded by MCIN/AEI/10.13039/501100011033, P20-00460, UMA20-FEDERJA-112 and FPU21/01318. University of Málaga.

Study of Plasma β-Amyloid, NfL, and GFAP as Potential Biomarkers for Monitoring Cognitive Impairment in Patients with Major Depressive Disorder

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Major Depressive Disorder (MDD) is a primary psychiatric disorder considered a risk factor for developing age-associated cognitive impairment (CI). Thus, to test this hypothesis, it is important to study CI biomarkers, particularly those related to Alzheimer's Disease (AD), such as β-amyloid protein (Aβ), whose most common forms are Aβ-40 and Aβ-42; neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP).

Traditionally, these peripheral biomarkers have been measured in cerebrospinal fluid, but the development of the Single Molecule Array (SIMOA) technique allows for the detection of very low concentrations of these biomarkers in blood. Therefore, our aim is to quantify plasma levels of these biomarkers in an MDD cohort compared to a healthy control (HC) group, within an age range of 20 to 75 years.

We expect to find alterations in these biomarkers in the MDD group compared to the HC group, following the pattern observed in CI, confirming that this disorder causes brain alterations that may lead to dementia (e.g., AD) or other types of CI in the future.

Therefore, demonstrating the validity of these peripheral biomarkers associated with MDD would allow for (1) reducing costs in the diagnostic process of CI (making it faster, more cost-effective, and less invasive for patients) and (2) implementing preventive measures or interventions to mitigate the effects of CI.

Studying the psychotic-like states induced by Delta-9-Tetrahidrocannabinol (THC): new behavioral strategies to tackle the complexity of psychosis in mouse models

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Cannabis is the most consumed illicit drug worldwide and its use has been associated with a major risk of developing psychotic symptoms such as positive psychotic-like states, as well as social and/or emotional skills impairment.
The neurobiology of positive psychotic-like states is poorly understood, partly due to a lack of more accurate animal behavioural paradigms. In the last years, complex cognitive processes such as higher-order conditioning have been proposed to partially model behavioral alterations linked to positive psychotic-like states. In rodents, higher-order conditioning is studied using sensory preconditioning paradigms where two low-salience stimuli are paired in a preconditioning phase, and then one of them is conditioned with a reinforcer. This results in a conditioned response to both the conditioned (direct learning) and the non-conditioned stimuli (mediated learning (ML)). Increasing the associations in the preconditioning phase, ML evolves into "reality testing" (RT), improving stimuli discrimination. The impairment of RT is considered a key feature of positive psychotic-like states.
In this study, we first set up a light-tone sensory preconditioning task in male and female mice and, interestingly, we develop a RT protocol increasing light-tone associations during the preconditioning phase of the task. Notably, an acute injection of Delta-9-tetrahidrocannabinol (THC) (1mg/kg) prior the ML test, impaired RT in male mice, indicating a disruption in the processing of mental sensory representations.
Furthermore, we assessed the impact of THC in social and emotional recognition, using novel tools for behavioral tracking that go beyond the traditional measures of social interaction.
In future steps, taking advantage of these behavioral approaches, we want to better understand the molecular mechanisms underlying the psychotic-like effects induced by THC, focusing on the possible role of mitochondrial cannabinoid receptor-1 and mitochondrial-related processes. Overall, we aimed at finding novel therapeutical targets that open new prospects for psychiatric disorders.

THE DUAL-HIT HYPOTHESIS OF SCHIZOPHRENIA: THC TREATMENT IN A MATERNAL IMMUNE ACTIVATION MODEL

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Schizophrenia is a neurodevelopmental psychiatric disorder affecting 0.6-0.8% of the global population. The prevailing theory suggests that both genetic and environmental factors contribute to its development. Strong epidemiological evidence supports an association between maternal infection during pregnancy and an increased risk of schizophrenia in offspring later in life. Additionally, cannabis use during adolescence has been linked to the onset of this disorder, leading to the formulation of the dual-hit hypothesis of schizophrenia. To assess this hypothesis, we propose an animal model that replicates both the cerebral and behavioral symptoms of the disorder. We have established a maternal immune activation mouse model using polyinosinic-polycytidylic acid (Poly(I:C)) in pregnant dams during gestation (GD9). In the postnatal period (PD 55-60), offspring exposed to Poly(I:C) are injected with delta-9-tetrahydrocannabinol (THC). The dual-hit model exhibits reduced body weight following THC exposure and presents phenotypes associated with positive symptoms in males, along with phenotypes linked to negative symptoms in both sexes. The proposed paradigm induces a reduction in Reelin levels, a biomarker of the disease, in the prefrontal cortex of THC-treated male mice compared to the dual-hit group, and in all three treatment groups relative to controls in females, alongside a decrease in the number of Reelin-positive cells in the same groups. Furthermore, the model demonstrates a reduction in cortical thickness in the THC-treated groups and a decrease in dendritic spine density in the prefrontal cortex and hippocampus in the dual-hit group. Additionally, disruptions in blood-brain barrier permeability and changes in glial markers are evaluated in the model. This animal model provides a valuable tool for investigating environmental theories and advancing our understanding and treatment of schizophrenia.

The effect of systemic ketamine on working memory history dependencies

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Working memory (WM) representations in primates are attracted to immediate prior stimuli (serial dependence) but repulsed from the modes in the long-term distribution of stimuli (history dependence). It has been shown that WM attractive serial dependence is reduced in people with schizophrenia or autoimmune anti-NMDAR encephalitis - diseases linked to hypofunctional NMDARs1,2, while people with dyslexia only show a reduction in history biases3. These distinct patterns across disorders offer insights into the neural and biophysical mechanisms underlying WM. Here, we study the mechanisms underlying WM serial and history dependence in four macaque monkeys performing a biased oculomotor delayed response task. In each session, stimuli followed a bimodal Gaussian distribution, with two diametrically opposed mode locations, which varied by session. We recorded the neural activity of prefrontal neural populations in two monkeys using acute recordings (128-contact Diagnostic Biochips probes). In some sessions, monkeys were administered ketamine, an NMDAR antagonist, systemically.
We used linear models to assess the serial and history dependence of the monkeys' responses. Surprisingly, monkeys exhibited mostly repulsive serial and history biases. Ketamine reduced repulsive serial dependence but increased repulsive history bias, suggesting distinct mechanisms for the two processes. Moreover, we used neural population decoders to predict the stimulus location from prefrontal neural activity and analyzed the relationship between the decoded locations and the behavioral errors. Our analyses indicate a link between prefrontal activity and the distinct mechanisms for serial and history biases. These findings have strong implications for attractor models that implement serial dependence1 and history effects4 based on biophysically plausible NMDAR-dependent mechanisms.

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The Neuromodulatory Effects of Transcranial Electrical Stimulation on Numerical Cognition: A Systematic Review with Meta-Analysis

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Numerical cognition refers to the ability to perceive and process quantitative and numerical information, serving as the foundation for mathematical development. Transcranial electrical stimulation (tES) techniques have been added to conventional neuroimaging methods in the study of the neurocognitive bases of numerical cognition. This integration aims both at basic science (establishing links between brain structures and cognitive functions) and applied research (evaluating potential cognitive enhancement effects).However, a comprehensive synthesis of the differential effects of specific stimulation protocols on numerical cognition is lacking.

A systematic review was conducted according to PRISMA Statement to analyze the effects of tES on numerical cognition in general. Additionally, a meta-analysis of the specific effects on arithmetic skills was carried out, both following a protocol registered in PROSPERO (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=274484).

The results of the systematic review (34 included studies) show that different tES protocols (direct current, alternating current, and random noise) applied over the intraparietal sulcus, angular gyrus, dorsolateral prefrontal cortex, and inferior frontal gyrus have neuromodulatory effects that translate into measurable changes in performance on numerical processing and arithmetics. This evidence, based on interference, confirms the involvement of these structures in numerical cognition.

The meta-analysis (12 included studies, 35 effects, 508 participants) showed that the effects of stimulation on arithmetic ability differ according to the stimulation protocol: most protocols (i.e. random noise, unilateral anodic and cathodic direct current stimulation) showed non-significant effects or had a small effect size, with high heterogeneity. The only protocol that showed a positive and highly significant effect on arithmetic ability, even supported by the low heterogeneity, was bilateral direct current stimulation. However, the small number of studies makes these results still provisional. In conclusion, the current evidence regarding the enhancing effect of tES on arithmetic skills is inconclusive, and further studies are needed to establish the therapeutic potential of this technique.

THE ROLE OF ADAMTS2 IN SCHIZOPHRENIA: VALIDATION IN A NOVEL TRANSGENIC MOUSE MODEL

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Schizophrenia is a complex multigenetic disorder that is typically diagnosed during adolescence or young adulthood. To investigate new genetic pathways related to schizophrenia, we have identified a set of six overexpressed genes in the peripheral blood of patients associated with the appearance of the disease (ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2 and UNC45B). After three months of treatment, the expression of these genes returns to normal levels. Among these genes, ADAMTS2 was the most overexpressed and appeared to be modulated by antipsychotics in responsive patients. ADAMTS2 is a secreted metalloprotease that significantly contributes to the N-t-cleavage and inactivation of Reelin, a schizophrenia biomarker. To further investigate the role of ADAMTS2 in schizophrenia, we have generated a knock-in mouse model that overexpresses ADAMTS2 in the forebrain. The overexpression of ADAMTS2 does not affect the genotypic frequency, mortality, or weight of the mice, and there are no differences between sexes or with age. The transgenic mice exhibit a clear sexual dimorphism in behavior, with males generally displaying phenotypes associated with positive and social symptoms, while females show phenotypes related to stress. The relationship between ADAMTS2 and Reelin has been confirmed, as a reduction in Reelin expression levels and accumulation of its N-R2 fragment were observed in the prefrontal cortex of the model.

The steroid sulfatase inhibitor STX64 improves cognitive deficiencies in murine models

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Aging produces in brain a progressive oxidative process and neuroinflammation that deteriorates cognition. These brain dysfunctions provoke high economic and social costs to developed societies. The research for treatments to prevent cognitive deficits associated with aging is a priority. Previous results suggest that STX64, a steroid sulfatase (STS) inhibitor, improves cognition in rodent models of neurodegenerative disease. In this work, we have designed cellular (immunohistochemistry), molecular (qPCRs with primers of inflammatory markers and cellular populations) and behaviour (mainly, novel object recognition test and passive avoidance for the study of learning and memory) assays to know if subchronic (6 months) oral treatment with STX64 rescues cognitive dysfunction present in aging mice. The mouse strain used in our studies was C57. Cognitive studies with novel object recognition have also been conducted using oral STX64 administration in female rats (Wistar strain) to test its anti-amnesic effects after acute administration of scopolamine. Our behavioural results showed that STX64 treatment improved cognition in aging mice, reflected as an improvement in object recognition and in passive avoidance tests. STX64 was also effective in the prevention of memory impairments induced by acute administration of scopolamine in rats. At cellular level, aging mice treated with STX64 showed an increase in microglial response and in hippocampal adult neurogenesis. Preliminary studies showed the C57 model of Alzheimer's Disease APP/PS1 also induced cholinergic activity in neurons after 1 month of oral treatment with STX64. The qPCR assays indicated that hippocampus from aging mice treated with STX64 express lower levels of inflammatory factors mRNA compared with untreated aging mice, though new studies are in progress to distinguish the microglial phenotypes of these animals using phenotype-specific primers. Then, our results indicates that STX64 may be an interesting drug for the treatment of cognitive decline associated to aging and/or neurological diseases.

The trifty phenotype: a key phenotype for mental health

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The intrauterine environment is essential for proper fetal development.
Adverse effects during this stage can lead to genetic modifications with negative consequences for the health of the offspring.
The "thrifty phenotype hypothesis" links fetal exposure to nutritional deficiencies with alterations in metabolism. This fetal malnutrition condition genetically prepares the fetus to face a presumed nutrient-poor environment. To achieve this, the glucose-insulin metabolism is permanently altered, increasing the risk of type 2 diabetes or heart diseases in the future. These metabolic alterations are reflected in poor intrauterine growth and abnormal postnatal growth. This abnormal development could be a target phenotype for detecting potential adverse intrauterine conditions.
A one-year study was conducted on 461 offspring from a cohort of mothers who gave birth during the SARS-CoV-2 virus pandemic. At 6 and 12 months of age, each child underwent a questionnaire assessing skills such as communication, gross motor skills, fine motor skills, problem-solving, and personal and social development (ASQ questionnaire). This cohort of offspring were divided into two groups:
Group 1: Babies who were born with low birth weight but had rapid weight recovery above one standard deviation.
Group 2: The rest of the babies.
Student's t-test revealed that, in problem-solving, Group 1 scored worse compared to Group 2.
These findings highlight the Developmental Origins of Health and Disease (DOHaD) model, which holds that factors influencing early development, both pre- and postnatally, can have lasting effects on physical and mental health throughout life.

Towards a dual learning system in humans: sensory and category information cause opposed biases in behavior

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Several studies in rodents and humans have shown that category information (frequency in which pieces of evidence favor a response option; CI) can increase primacy and confirmation biases, whereas sensory information (stimulus strength towards the correct option; SI) can do the opposite. Guided by a new qualitative modular learning model, our behavioral experiment in humans aimed to show that CI and SI can modulate task accuracy through changes in integration biases and degree of caution.
59 healthy university students (86% females) performed two decision-making tasks. In Clouds-of-Dots trials, participants identified the dominant color of a sequence of 10 static dot clouds (red or blue) with a right-left bottom press. Then, they rated their response confidence (1–4 Likert scale). Correct responses earned virtual coins. In Preference trials, participants were shown five pairs of images (e.g., animals, fruits). The first four showed the preferences of an unknown player (e.g., orange > apple, …). In exchange for extra coins at the end of the experiment, participants had to infer the preferred stimulus at the fifth pair. Immediate feedback only included the true preference.
As expected, higher SI increased accuracy ($p<.001$, $r=.87$). However, strikingly, low CI increased accuracy ($p<.001$, $r=.87$) and confidence ($p=.01$, $d=.27$), while decreasing reaction time ($p=.019$, $d=.35$). The accuracy improvement tended to be stronger in cautious participants (more negative metacognitive bias; $p=.07$). Moreover, low CI tended to increase caution ($p=.07$, $d=-.27$), while low SI increased it ($p<.001$, $d=2.16$). On the other hand, 42% of participants showed integration bias patterns (e.g., U-shaped kernels) that have not been predicted by previous models. In conclusion, each information type seemed to have opposite effects in deliberation caution, and cause a higher diversity of integration bias patterns than previously found or predicted. A dual model of learning may be needed to account for these results.

Tracking miRNA signatures over 10 years in Multiple Sclerosis

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Multiple sclerosis (MS) is characterized by inflammation, demyelination, and neurodegeneration of the central nervous system (CNS). MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression and have been implicated in MS. Due to their stability in biological fluids, miRNAs are promising biomarkers for prognosis and personalized treatment. This study evaluates miRNA expression longitudinally in MS patients at baseline and 10 years follow-up to assess their potential as predictive biomarkers of disease progression.
Buffy coat samples from 34 MS patients with a confirmed diagnosis were collected at baseline and after 10 years follow-up. miRNA were extracted and analyzed using custom 384-well Taqman Gene Expression Array Cards, focusing on 31 unique miRNAs. Expression changes were determined using the ΔΔCt method and protein target analysis was performed using open-access databases. Data were analyzed using appropriate statistical tests based on normality, and statistical significance was set at $p<0.005$. Significant differences in miRNA expression were identified over the 10-year follow-up period. Significant differences were observed in miR-188-5p, miR-197-3p, miR-199a-5p, miR-181c-5p, miR-119b-3p, miR-30c-5p and miR-335-5p ($p=0.031$; $p=0.002$; $p=0.001$; $p=0.017$; $p= 0.043$; $p=0.007$; $p=0.011$), respectively. These miRNAs have been found to target key proteins involved in immune regulation, apoptosis, and neuroinflammation, such as MAPK1, ETS1, FOXO3, RUNX3, and TNFSF10. The observed changes in miRNA expression over 10 years in MS patients suggest their possible involvement in disease progression, particularly in neuroinflammatory and neurodegenerative processes. Identifying these miRNAs as potential biomarkers could enhance our understanding of the disease progression and the molecular changes that develop over time.

Visceral Adipose Tissue as a Mediator of Metabolic Dysfunction and Tau Pathology in Alzheimer's Disease

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Alzheimer's disease (AD) is a complex disorder involving multiplecellular and molecular mechanisms. Recent evidence suggests that metabolicalterations play a crucial role in AD progression. Likewise, diabetes and obesity—two major metabolic diseases—are well-established risk factors for AD. Theseconditions are associated with a significant expansion of visceral adipose tissue.Here, we hypothesize that visceral adipose tissue may act as a key mediatorbetween peripheral metabolic dysfunction and brain illnesses.

We used histological stains, immunohistochemistry, and biochemicalassays to analyze changes in visceral adipose tissue from WT, db/db, and WTmice subjected to a high-fat diet (HFD). Additionally, we performed adipose tissuetransplants from db/db donors into 3xTg-AD mice and from HFD-fed WT donorsinto Tau P301S mice to evaluate potential alterations in tau pathology in thesedistinct AD models.

Our study shows that 3xTg-AD mice receiving fat pads from db/db donorsexhibited a significant increase in tau pathology, driven by elevated p25/p35expression, compared to 3xTg-AD mice receiving fat from standard-diet WT mice.This tau accumulation was associated with increased IL-1β levels and robustmicroglial activation. Similarly, Tau P301S mice transplanted with fat from HFD-fedWT donors also displayed exacerbated tau pathology and neuroinflammation,indicating that metabolic dysfunction induced major changes in neuroinflammationthat contributed to tau pathology.

Overall, our study highlights a novel interplay between AD andmetabolic disorders, demonstrating that visceral adipose tissue from bothgenetically obese/diabetic and diet-induced obese mice exacerbates tau pathologyvia immune system activation. These findings reinforce the role of metabolicdysfunction in AD and suggest visceral adipose tissue as a potential target fortherapeutic intervention.

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

INDEX

A prediction circuit for deviant stimulus detection signals error direction in primary somatosensory cortex

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The predictive coding theory proposes that the cerebral cortex generates internal models of the world to anticipate incoming sensory inputs and then produces prediction error (PE) signals when discrepancies arise, which further optimize the internal model. Nonetheless, the basic neuronal circuit motif that performs this computation has not been fully identified. In this study, we recorded neuronal activity of layer 2-3 excitatory neurons by using in vivo 2-photon Ca2+ imaging in vibrissal primary somatosensory cortex. We implemented a prediction paradigm by stimulating whiskers in three different contexts: (1) a highly predictable protocol where a sequence of repetitive whisker deflections of the same low amplitude was randomly interrupted by an unexpected (10% probability) deviant stimulus of higher amplitude; (2) the reverse protocol, where the low amplitude stimulus became the deviant within a background of repetitive stimuli of higher amplitude; (3) a non-predictable control protocol where stimuli of 5 different amplitudes (including the previous amplitudes) were randomly presented with the same probability. At a population level (1.152 whisker-responsive neurons, ~45% of all recorded neurons), neuronal responses for a given amplitude were markedly different depending on the predictability context. For both low and high amplitude stimuli, the responses elicited when they were repetitive (i.e., predicted) were smaller than in the control context, consistent with sensory adaptation. However, responses to deviant stimuli differed greatly: responses to the higher amplitude deviant were significantly larger than in the control context, while responses to the lower amplitude deviant were smaller. Our results are consistent with a simple computation in which the response of PE neurons is the difference between the actual sensory stimulus and its prediction. Thus, a sensory input that elicits a higher response than the expected input is amplified by PE neurons, while a stimulus that elicits a lower-than-expected sensory input is suppressed.

A Theoretical Framework for Criticality and a Hierarchy of Timescales in the Brain

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Brain criticality is often studied in terms of phase transitions, typically assuming a single control parameter. However, neural systems exhibit inherent heterogeneity, with diverse excitability at the microscopic level giving rise to multiple percolations and transitions. At the macroscopic level, this heterogeneity supports a hierarchy of timescales, ranging from rapid neural responses to slower cognitive processes. A key question is how brain criticality, which emphasizes phase transitions, aligns with the observed hierarchy of neural timescales. We developed a theoretical framework integrating nonlinear dynamics and criticality theory to examine the relationship between hierarchical timescales and proximity to criticality. Specifically, we analyzed how critical slowing down—where systems near a phase transition exhibit prolonged recovery times—shapes neural timescales. Using existing empirical findings on functional brain hierarchy and criticality, we explored how regions with slower timescales align with these principles and contribute to balancing sensitivity and stability in neural processing. Our analysis suggests that brain regions are positioned at varying distances from criticality. Regions with slower timescales tend to operate closer to the critical point due to critical slowing down, while regions with faster dynamics function in subcritical regimes. This structured coexistence of critical and subcritical dynamics enables both sensitivity to external stimuli and reliable internal processing, naturally giving rise to a hierarchy of timescales. By integrating criticality and hierarchical timescales, this framework provides a novel perspective on brain organization. It suggests that brain regions exist along a continuum of criticality, with functional requirements shaping their dynamic properties. This model offers insights into neural computation, cognition, and potential implications for neurological disorders.

Aging and Brain Criticality: Diverging Pathways of Neural Dynamics

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Brain criticality—the balance between stability and flexibility in neural activity—is essential for cognition and adaptability. However, how aging affects criticality remains debated. Some studies suggest normal aging shifts neural dynamics toward subcriticality, reducing variability and leading to cognitive slowing. Others propose aging may push the brain into a supercritical state, increasing hyperexcitability and instability.

To reconcile these perspectives, we developed a neuronal network model simulating aging as two parallel processes: healthy aging, which steadily prunes network connections, and pathological aging, which introduces random lesions that locally alter excitability. We tracked the brain's distance to criticality using temporal correlation length (intrinsic timescales).

Our findings reveal two distinct aging trajectories.
In normal aging, neural dynamics shift toward subcriticality, aligning with observed declines in neural variability and cognitive speed. In contrast, pathological aging initially reduces criticality due to network degradation but later drives the system toward supercriticality, potentially leading to hyperexcitable states seen in neurodegenerative diseases. This transition occurs as accumulated lesions trigger compensatory excitability changes, disrupting the neural balance.

Our model unifies conflicting findings, demonstrating that normal and pathological aging follow distinct criticality pathways. These insights provide a theoretical framework for understanding neural aging and highlight the potential for early detection of neurodegenerative diseases. By identifying mechanisms driving shifts in brain criticality, our work suggests new avenues for interventions aimed at preserving stable neural dynamics in aging populations.

Balanced inhibition allows for robust learning of input-output associations in feedforward networks with Hebbian plasticity

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In neural networks within the brain, the activity of a post-synaptic neuron is determined by the combined influence of many pre-synaptic neurons. This distributed processing enables mechanisms like Hebbian plasticity to associate sensory inputs with specific internal states, as seen in feedforward structures such as the CA1 region of the hippocampus. By modifying synaptic weights through Hebbian rules, sensory inputs can subsequently elicit outputs that reliably correlate with their associated internal states. When input and output patterns are uncorrelated, this approach allows for the encoding of a large number of distinct associations, enabling efficient memory storage.

However, our study demonstrates a critical limitation when output patterns become weakly correlated with input patterns, such as through the intrinsic feedforward connectivity of the network. In these cases, the Hebbian rule preferentially strengthens synaptic weights shared across patterns, leading to a "freezing" of the network's structure. This results in highly correlated output patterns over time, effectively reducing the network's capacity to store diverse associations and limiting its flexibility in learning.

To address this challenge, we find that including a mechanism of balanced inhibition, which has been shown to be a key feature of cortical circuits in-vivo, counteracts the undesired correlations between inputs and outputs. By dynamically regulating inhibitory input, balanced inhibition prevents the over-strengthening of shared weights, restoring the network's ability to maintain robust and flexible learning. This finding underscores the importance of inhibitory mechanisms in enabling efficient and adaptive information processing in neural circuits, offering insights into how biological networks maintain their remarkable capacity for associative learning.

Computational investigation of temperature modulation in cortical dynamics

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The cerebral cortex exhibits a rich repertoire of spontaneous spatiotemporal patterns of activity that depend on the brain's dynamical regime. These dynamics can range from highly synchronized (e.g., slow wave sleep) to asynchronous patterns (e.g., wakefulness). During NREM sleep, cortical activity is characterized by slow oscillations (SO) where neuronal circuits spontaneously switch between periods of neuronal firing (Up states) and silence (Down states) at approximately 1 Hz [1]. Conversely, during awake states, the cerebral cortex exhibits asynchronous activity, with a suppression of the synchronized low-frequency content. However, under specific conditions, slow waves can spontaneously emerge and propagate within awake cortical regions, such as in cases of brain lesions [2]. Particularly, SO generation around the lesion co-exist with a localized increase in temperature. Under this framework, we aimed to quantitatively explore how perilesional SO generation and wave propagation into desynchronized cortical areas can be modulated by temperature.

We employed a biophysically realistic two-dimensional network model reproducing SO and desynchronized activity observed in vitro cortical slices [3]. First, we explored how manipulations of cellular and synaptic parameters affected by temperature could explain in vitro experimental observations [4]. Using the desynchronized model, we then induced SO in a localized cortical area to examine how temperature may modulate SO generation and propagation into desynchronized regions. Our results indicate that increased local temperature enhances synchronization, promoting SO generation and percolation into desynchronized regions. These findings suggest that temperature increase may play a role in perilesional SO generation and represents a step toward understanding the dynamic changes occurring in the lesioned brain.

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Computational models show that Feedforward and Feedback Inhibition Flexibly Modulates Theta-Gamma Cross-Frequency Interactions in Neural Circuits

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Brain rhythms coordinate neuronal activity, with cross-frequency coupling (CFC) between theta (~8 Hz) and gamma (~30 Hz) being crucial for memory. While traditionally slow oscillations were thought to modulate faster ones, new metrics like cross-frequency directionality (CFD) reveal bidirectional influences. This study presents a computational circuit model that flexibly displays both directions of interaction, depending on the balance between inhibitory sub-motifs.

We analyzed two motifs based on Pyramidal-Interneuron Network Gamma (PING/ING) models, where interactions between pyramidal cells (PCs) and inhibitory basket cells (BCs) generate gamma rhythms. An external theta drive modulated network activity, inducing cross-frequency interactions. Our model was validated with a dataset analyzing Entorhinal Cortex layer III (ECIII) projections to hippocampal CA1 in rats navigating different environments.

Our analysis revealed that in θ-ING motifs, feedforward recruitment of BCs drives gamma-to-theta directionality (CFD<0), while in θ-PING motifs, feedback inhibition favors theta-to-gamma directionality (CFD>0). In combined motifs, varying synaptic strengths within realistic ranges, we found smooth transitions between directionalities. Experimental data showed that behavioral conditions modulated CFD and gamma frequency in line with our model predictions. Finally, by evaluating each motif's capacity to integrate distinct inputs impinging at different sites of the PC dendritic tree, we report their differential role in prioritizing transmission across different information channels.

Our framework suggests that feedforward/feedback inhibitory balance regulates the directionality of theta-gamma interactions. Notably, θ-ING/θ-PING modes exist along a continuum rather than as distinct alternatives. In our model, CFD analysis identified transitions between functional modes, aligning with experimental observations across different behavioral states. Finally, we showed that a feedback-shifted balance promotes strong afferent-driven cross-frequency rhythmicity, while a feedforward-shifted motif broadens encoding windows, favoring parallel pathway transmission. Thus, dynamic CFD measures may reflect predominant inhibitory motifs and flexible prioritization of functional connectivity pathways.

Customizable computational model of rat brain functional connectivity

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We develop a customizable computational model to replicate functional interactions between rat brain regions. The model simulates brain activity based on structural data through advanced diffusion-weighted (dw) MRI, which included measured length and average conduction velocities of individual nerve tracts. Personalized brain computational models can be crucial for advancing neuromodulation approaches, as these techniques often face limitations due to individual variability in brain structure and function.

The model simulates functional connectivity (FC) at the population level using coupled Stuart-Landau subcritical complex oscillators (Cabral 2022). We use MRI data from 60 rat datasets to extract anatomical features and structural connectivity metrics across 152 brain regions (ROIs), including connection weight (fiber bundle size), axonal length, density, diameter, and myelin thickness. These measures enable the calculation of communication delays between different ROIs, both at the group and individual levels, representing a significant advance in whole-brain FC models. These metrics are captured in 152x152 matrices that drive our Stuart-Landau model, generating neuronal time courses, from which synthetic BOLD signals are derived. By correlating these signals across ROIs, we create FC matrices.

Preliminary results of simulations using individualized connection delays result in distinct FC patterns between ROIs, compared to traditional methods that do not account for conduction velocity variability. Our approach generally reveals stronger functional connectivity between ROIs. We demonstrate how axonal conduction velocities influence structural connectivity, providing insights through complex network measures applied to the metrics driving our simulations.

Introducing conduction velocity in computational brain models alters FC matrices. In ongoing work, we compare the computational matrices with FC matrices derived from actual BOLD signals obtained from the same ROIs in the same animals, using information theory-based methods to assess their potential relative to existing approaches.

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Emergence of sequential dynamical invariants in chaotic winnerless competition neural networks

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Neural sequences play a critical role in both behavior and cognitive function, balancing stable sequential ordering with flexible timing to allow effective adaptation. Analyzing neural cyclic activation timings reveals constraints shaping sequence intervals and limiting flexibility. Prior studies identified these constraints as dynamical invariants —consistent temporal relationships between intervals that build the sequence— in bursting activity of central pattern generators. However, dynamical invariants and their role remain largely unexplored in computational models. This study examines dynamical invariants in a computational network displaying winnerless competition dynamics, which is known for generating chaotic but well-structured neuronal activation sequences.
Here we employed a computational neural network modeled with Lotka-Volterra differential equations characterized by chaotic heteroclinic dynamics arising from asymmetric neuronal connections. Each equation represents the spike rate of the neurons. To systematically identify sequential events, the neuron with the most consistent activity was selected as a temporal reference within each cycle. Activation events in other neurons were detected using a threshold criterion, defining intervals as the time between activation and the subsequent deactivation of neurons. Interval relationships were examined using statistical variability measures, correlation analyses, and principal component analysis.
Despite the intrinsic chaotic dynamics useful for exploratory cognitive and motor behaviors, robust dynamical invariants emerged consistently between specific time intervals. These invariants reinforce temporal coordination among neurons, suggesting an inherent constraining mechanism to the variability present in the chaotic activity. This study is the first to identify sequential dynamical invariants within a network exhibiting heteroclinic dynamics.
Dynamical invariants have rarely been studied beyond minimal central pattern generator circuits, largely due to lack of variability observed within model neurons and network dynamics. The winnerless competition model, however, produces chaotic spatiotemporal patterns, effectively overcoming this limitation. Our results suggest that dynamical invariants are part of the sequence coordination mechanisms of the heteroclinic dynamics.

Emergent Representational Stability from Unstable Neuronal Activity: Modeling Representational Drift

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Neurons in the brain show ongoing changes in their response properties over days, even under behaviorally and environmentally stable conditions - a phenomenon termed representational drift. This drift has been documented across multiple brain regions, including motor cortex (Rokni et al., 2007), hippocampus (Ziv et al., 2013), and auditory cortex (Aschauer et al., 2022). Although this instability at the level of single-neuron activities appears incompatible with stable percepts and behavior, recent analyses suggest that neuronal representations can remain remarkably stable at the global level (Gallego et al., 2020; Noda et al., 2024).

Here, representations are understood as "representations of similarities," where the relatedness between stimuli is encoded in the similarity of the corresponding neuronal activity patterns (Edelman, 1998). Together, these global activity patterns define a structured representation in which the relationships between stimuli are preserved. This preservation of representational structure despite ongoing changes in individual neuronal responses raises a fundamental question: How do neuronal circuits maintain stable representational geometry when their constituent elements are in constant flux?

To address this paradox, we propose that statistical learning - the continuous extraction of regularities from sensory experience to infer structure in the environment - may provide a key mechanism for maintaining stable representations. Utilizing a recurrent neuronal network model, we systematically investigate several fundamental questions: How can network dynamics capture the geometric relationships between stimuli? What mechanisms maintain these relationships during ongoing drift? And critically, how can the network preserve stable representations while remaining plastic enough to support ongoing learning of stimulus regularities?

We explore whether input correlations alone are sufficient or if additional plasticity mechanisms, such as Hebbian learning, are necessary to guide the ongoing changes in neuronal activity. Our theoretical framework aims to provide mechanistic insights into how neuronal circuits achieve global stability despite local variability.

Fine-tuning of attractors underlies the learning of task robust working memory in mice

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Working memory (WM) is a process that temporarily holds information when not accessible to the senses. It relies on storing stimulus features in neuronal activity. Despite its importance in cognition, WM is quite vulnerable due to its limited capacity and stability, especially when facing interferences from internal and external sources. Recent studies have explored the neural mechanisms that protect WM and proposed that pre-/post-distraction neural activity decomposes into orthogonal subspaces, thus protecting information. However, whether this generalizes to competing WM items or competing tasks is unclear. Here, we study the learning of concurrent WM representations using calcium imaging data from the mouse prelimbic (PrL) and anterior cingulate (ACC) cortices as they learn an olfactory dual task. The task combines an outer Delayed Paired-Association task (DPA) with an inner Go-NoGo task. We examined how neuronal activity reflected the process of protecting the DPA sample information against the Go-NoGo task. As mice underwent learning, we measured the overlap between the activity and the low-dimensional subspaces that encode sample odors and animal choices (lick/no-lick). Early in the learning, post-Go-NoGo activity overlapped with the lick region of the sample/choice subspace. Later in the learning, post-Go-NoGo activity was confined to the no-lick region. We present a mechanistic insight into these results with recurrent neural network models with trained low-rank connections. Models were trained on the DPA, on the Go-NoGo, and finally on the dual task. The models link (1) pairing of the DPA sample and test odors to a rotation in the sample/choice plane and (2) learning to the fine-tuning of a double-well attractor in that plane. We validated our predictions by estimating energy landscapes for the fixed points of the recorded neural dynamics. In sum, our study underscores the crucial role attractor dynamics plays in shielding WM representations from concurrent tasks.

Impact of age-related perturbations on a bump attractor model of working memory

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Normal aging in humans and non-human primates is associated with progressive cognitive changes, which are especially evident in working memory tasks. Working memory relies on the dorsolateral prefrontal cortex, that is undergoing pronounced alterations during aging, including myelin loss, synapse loss, and neuronal hyperexcitability. Despite a wealth of experimental data, a coherent theoretical framework of how these age-related neuronal changes interact and alter network dynamics is currently lacking. Here, we investigated how both the individual and shared contributions of aging factors lead to working memory decline in bump attractor networks of working memory.

The spiking neural network incorporates two key aging factors: myelination deficits and neuronal hyperexcitability. It consists of excitatory and inhibitory populations of leaky integrate-and-fire neurons with sparse connectivity, that also incorporate short-term synaptic depression and facilitation (Hansel and Mato, 2013). Myelin loss was modeled as an increase in the action potential failure rate (Ibañez et al., 2023), and changes in excitability were introduced by modifications to the f-I curve of leaky integrate-and-fire neurons, fitted to empirical data (Ibañez et al., 2020).

Our models predict that biologically plausible levels of myelin loss and hyperexcitability account for substantial working memory impairment with aging, although via distinct mechanisms. Hyperexcitability leads to a spread of activity among neurons and increased neuronal correlations within the network. Consistent with theory, these changes in the network dynamics lead to increased diffusion of the activity bump and therefore predict less precise working memory representations. In contrast, myelin loss severely impacts the amplitude but not the width of the activity bump, resulting in reduced firing rate levels and stability over time, thereby primarily impairing memory duration.

These findings highlight the different impacts of age-related changes on working memory circuit functionality, providing insights into the mechanisms of cognitive decline along with potential pathways for prevention and treatment.

Modeling V1-LM Cortical Interactions: A Data-Driven Approach to Visual Processing in the Mouse Brain

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We present a biologically realistic computational model of the lateral medial (LM) and primary visual (V1) cortical areas of the mouse brain, designed to investigate their roles in visual processing. By integrating topographically-driven inter-area connectivity from Reimann et al. (2019) with the detailed V1 cortical microcircuitry from Billeh et al. (2020), we developed a high-fidelity V1-LM model that captures neuronal composition and synaptic organization. Our model employs an innovative bidirectional approach, combining structural connectivity data with functional Neuropixels recordings, enabling biologically constrained deep learning training in TensorFlow to reproduce key cortical properties, including firing rates, orientation and direction selectivity, and synchronization.

This model provides insights into how LM modulates spatial information processing and dynamically interacts with V1 to shape visual computations. Our results demonstrate that LM influences not only the receptive field properties and response latencies of V1 neurons but also enhances computational capabilities such as robustness and high-dimensional feature separation in classification tasks. By bridging anatomical structure with emergent function, this work contributes to a deeper understanding of hierarchical visual processing and the role of secondary visual areas in sensory integration.

On the Implications of Nitric Oxide Diffusion for Hebbian Learning. A New Expression of Hebb's Law

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From a computational perspective, the brain is a massively parallel system driven by functional coupling of neurons, cell communication, and learning. Computational neuroscience aims to understand brain computing using simple and realistic computational models.
In this work, we study the learning according to Hebb's law, using a system of nonlinear differential equations called the Hebbian System (HS).
We propose a scheme to explain the modification of neuronal connections through a new type of cell signalling based on nitric oxide (NO) diffusion, the volume transmission (VT). We incorporate VT into HS using our multi-compartment NO diffusion model and we investigate whether NO conditions neuronal connection modifications.
We present an HS based on Grossberg's INSTARs and OUTSTARs. We condition the dynamic of NO on the activation states of interneurons and the dynamics of learning on that of NO. In this way we obtain the Diffusion Hebbian System (DHS), where NO diffusion carries information from sensory neurons to interneurons.
The results indicate the role of NO as a biological support for retrograde communication in Hebbian learning, as there is information retrieval with DHS dynamics, albeit with a mean MSE of 24.68%, compared to 16.23% for HS systems, and convergence of the Mean Squared Error for INSTAR and OUTSTAR with DHS dynamics, higher than for HS dynamics.
We have reached important conclusions about the effect of the VT by the NO, on Hebbian learning, in both biological and artificial neural networks. We have confirmed the establishment of a new and more general framework of neural learning, which is based on synaptic and diffusive terms.
We have showed that the NO diffusion effect in a Hebbian neural system can be an underlying mechanism for supporting the Hebb's learning law, revealing the capacity of NO diffusion for the transmission of neural information.

Structural determinants of high-order interactions in biophysical neural networks

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High-order interactions represent a novel data-driven and principled approach to study brain dynamics beyond pairwise analyses, enabling a refined characterization and prediction of aging, neurodegenerative diseases, and states of consciousness. By considering groups of brain regions as functional units, these interactions reveal not only the strength but also the quality of information sharing, distinguishing between synergy—where the whole contains unique information that cannot be obtained by observing the parts alone—and redundancy—where information is repeated between regions. Although recent studies indicate that synergy-dominated interactions are widespread in the brain, their structural determinants remain unclear.
This study investigates how structural connectivity influences the emergence of high-order interactions, particularly those dominated by synergy, through the use of biophysically realistic neural mass models. We employed both synthetic and empirical structural connectivity matrices. Synthetic connectomes were constructed with three disjoint modules indirectly connected via a rich club, while the empirical connectome was derived from macaque anatomical data (CoCoMac). Multivariate information theory, particularly the O-information metric, was used to measure the dominance of synergy (negative values).
Our findings demonstrate that in synthetic networks, synergy-dominated interactions primarily arise from inter-module interactions mediated by indirect connections. Similarly, in the empirical connectome, such interactions often involve regions from distinct functional networks. Moreover, the degree of synergy dominance was modulated by the presence of direct connections; stronger direct connectivity diminished synergy by increasing synchronization. These results are consistent with previous observations that associate redundancy with positive functional correlations and synergy with both positive and negative correlations. Overall, the study sheds light on the mechanisms through which high-order interactions emerge in the brain, offering a more grounded interpretation on their potential as key indicators of brain states and health.

The Cosmic Brain project

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The Cosmic Brain project aims at exploring the application of analysis tools developed for cosmology to the modelling of the neural network and the connectome in the human brain. It exploits the striking similarities between the structure of the brain and the cosmic web. In particular, we will use the fact that both the cosmic web and the connectome emerge as self-organizing systems starting from noisy states to apply mature and well-tested techniques in cosmology to model the latter's structure. As a showcase, we present applications of the cosmological webon code applied to MRI data from the OASIS-3 database to obtain regions of interest which are highly correlated with brain aging. We also present an application of sonification for inclusion of visual impaired persons. Our proposed interdisciplinary methodology has a potential impact on the early diagnosis of neurodegenerative brain diseases such as Alzheimer and Parkinson.

Theoretical constraints on memory storage in models of hippocampal CA3

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The hippocampus is critical for learning and memory. It contains the largest auto-associative network in the brain, the CA3 recurrent collateral network, which enables high-capacity memory storage. However, despite decades of research, how hippocampus learns remains elusive. Moreover, how synaptic and circuit properties relate to hippocampal function in different species remains an open question.

Here, we establish a CA3-like Hopfield-type model [1] that is able to learn and recall patterns [2,3] to determine the optimal network characteristics leading to maximal memory capacity for networks of different sizes. To account for different brain sizes, we applied an analytical approximation, which allows us to compute storage capacity for realistic neuronal numbers [2,4]. To evaluate how distinct properties optimize memory storage, we studied networks with a fixed number of total synapses ranging from large sparse networks to smaller densely connected ones. We find that, regardless of the total number of synapses, arranging the same number of synapses in a larger, more sparsely connected network results in a >4 fold higher memory storage capacity.

We confirmed these findings using a Bayesian inference approach that assesses model parameters in an unbiased manner [5]. Together, these results suggest that an evolutionary drive for improved memory capacity would prioritize an expansion of neural number over an increase in network connectivity. These properties are consistent with direct measurements of CA3 circuit architecture and neuronal morphology across species, where connection probability in CA3 is lower in mouse than human [6].

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Understanding Microscale Diffusion in the Live Brain Extracellular Space

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The extracellular space (ECS) is a highly complex space composed of narrow interconnected channels and reservoirs. The ECS is a dynamic structure which is emerging as an important regulator of brain functions such as metabolite clearance and cell signalling. Still, it remains unknown how and to what extent, the ECS and its dynamics regulate these functions. The main limitation to understand local diffusional properties of the live brain ECS is the difficulty of current techniques to reconcile submicron resolution with live-tissue compatibility. Super-Resolutions Shadow Imaging (SUSHI) technique overcomes this limitation and allow us to optically resolve the nanoscale structure of the ECS in live brain tissue [1].

Accordingly, we have established a numerical computational model of diffusion in live tissue based on SUSHI images. This model is a novel tool to investigate 3D diffusion of different molecules at different spatio-temporal scales. As a first step, we developed a pseudo-3D model to simulate 3D diffusion in a more cost-efficient way. Then we compared and validated the SUSHI-based model against well-established experimental and computational methods to investigate diffusion. Finally, we have applied our model to understand how the nanoscale ECS geometry shapes the diffusion of the principal neurotransmitters (Glutamate and GABA) in different biological contexts.

To conclude, the proposed computational is a novel tool that can be applied in a variety of different biological contexts. More particularly, this work facilitates new insights into the role of ECS structure in diffusion and cellular signalling on the sub-micron scale, which still remains a poorly understood phenomenon.

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DISORDERS, NERVOUS SYSTEM REPAIR AND HEALTHY AGING

A novel mouse model to study TDP-43 autoregulation and its implication in the pathogenesis of amyotrophic lateral sclerosis (ALS) and other TDP-43 proteinopathies

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TDP-43 is a highly conserved RNA-binding protein, involved in multiple steps of mRNA processing and maturation. Extensive evidence has suggested that alterations in TDP-43 homeostasis play a central role in the pathogenesis of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases, which have been collectively named TDP-43 proteinopathies.

TDP-43 is extremely sensitive to changes in its expression levels and exhibits a complex autoregulatory mechanism to maintain physiological expression levels and avoid toxicity, which implies the binding of TDP-43 protein to a region on its own mRNA 3'UTR called TDP-43 binding region (TDPBR). However, the possible role of the autoregulation process in the pathogenesis of TDP-43 proteinopathies is still unknown. We have recently generated a new mouse strain with a conditional deletion of the TDPBR region critical for the control of autoregulation. This model will allow us to study the role of the TDPBR region in autoregulation both in vitro and in vivo, as well as the underlying molecular mechanisms in relation to the pathogenesis of ALS and other TDP-43 proteinopathies.

Accumulation of PFAS in the myelin sheath: Long-term consequences on myelin stability physiology and functional remyelination stability

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Over the past 30 years, an unexplained increase in the incidence of multiple sclerosis has been observed in developed countries, with a suspected contribution from environmental factors. We wondered whether exposure to some endocrine disrupting chemicals, in particular fluorinated surfactants (PFAS) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), could interfere with myelin formation and remyelination. Our hypothesis is that PFAS could accumulate in lipid-rich structures such as myelin, thereby disrupting myelin sheath integrity. Accordingly, we have recently demonstrated that PFAS accumulate in the myelin sheath of offspring exposed via maternal drinking water during late gestation and lactation. Furthermore, using ex vivo and in vivo approaches, we have shown that PFOS, but not PFOA, impaired functional remyelination. Here, we investigated the long-term effects of perinatal PFOS exposure on oligodendrogenesis, myelination and remyelination. First, we showed that perinatal PFOS exposure impaired neurogliogenesis in the adult subventricular zone and blocked oligodendroglial maturation in the corpus callosum. Transmission electron microscopy studies in the corpus callosum showed that PFOS permanently reduced myelin sheath thickness and altered myelin sheath ultrastructure, suggesting permanent changes in myelin integrity and stability. To analyse this hypothesis, we developed an in vitro system based on the production of myelin-like giant unilamellar vesicles, which allows us to assess how PFOS exposure might affect the mechanical and biophysical properties of myelin. By using this method, we showed that the addition of PFOS led to a weaker and more fragile myelin lipidic bilayer. This result was also confirmed by in silico membrane dynamics simulations.
In conclusion, our data show that perinatal exposure to PFOS, and to a lesser extent to PFOA, results in permanent changes in myelin physiology that may increase susceptibility to myelin disorders such as multiple sclerosis.

Activation of NOTCH pathway in brain endothelial cells ameliorates vascular abnormalities in Alzheimer's disease mouse models

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In previous studies, we identified low-perfused vascular abnormalities, termed "vascular scars", surrounding Aβ plaques in Alzheimer's disease mouse models. We hypothesized that these vascular scars result from non-productive angiogenesis, a process in which total or partial reduction of the DLL4/NOTCH pathway leads to erratic angiogenesis and disassembly of vessels into non-conducting structures. In this study, our objective was to investigate whether restoring the NOTCH pathway in brain endothelial cells could enhance vessel stability around Aβ plaques. To this aim, we injected the adeno-associated viral vector AAV2-BR1 in the tail vein of 5-month-old wild type and APP751SL/+ or APP-PSEN1/+ mice. This vector targets brain endothelial cells and delivered NOTCH intracellular domain 1 (NICD1), a key signaling factor in the DLL4/NOTCH pathway. After two months, we performed histological and biochemical analyses on the collected brains. Quantitative RT-PCR confirmed the specific expression of NICD1 in AAV2-BR1-NICD1 mice compared to control mice. Immunofluorescence imaging revealed an increased presence of vascular markers near Aβ plaques in APP751SL/+ mice expressing NICD1. Remarkably, both APP models exhibited improved performance in memory tests when injected with NICD1, indicating partial rescue of pathology-associated cognitive deficits. To further elucidate the consequences of NOTCH pathway activation on vessel stability, we are now exploring genomic expression of endothelial and microglial cells in APP751SL/+ vs APP751SL/+-NICD1 mice. Altogether, these findings position NOTCH pathway activation as a promising approach for ameliorating vascular abnormalities associated with Alzheimer's disease, offering insights into potential therapeutic avenues.

Addressing mitochondrial dynamics in intercellular transfer to glioblastoma

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Glioblastoma multiforme (GBM) is a recurrent central nervous system cancer with a poor prognosis that remains incurable, primarily due to its resistance to the limited range of available therapies. This resistance has been shown to be mediated by mitochondrial content and function, which are largely regulated by mitochondrial dynamics. These dynamics result from the balance of fission events controlled by Drp1 and fusion events tightly regulated by Mitofusins (Mfn1/Mfn2) and Opa1, which are key players in the outer and inner mitochondrial membranes, respectively. Notably, mitochondrial dynamics have been expanded by the recent discovery of their ability to be transferred intercellularly, leading to a content and metabolic reconfiguration in GBM that primes tumor development.

To investigate whether and how fusion and fission regulates transfer, here we aimed to determine whether the genetic ablation and individualized pharmacological modulation of mitochondrial shaping proteins affect transfer to GBM cells. Analysis of mitochondrial DNA (mtDNA) and tracking of whole mitochondria acquisition revealed that GBM cells can import free mitochondria from the extracellular medium, as well as mitochondria transferred through intercellular membranous bridges termed tunneling nanotubes (TNTs). Capitalizing on concomitant or time-differentiated kinetic approaches, we observed that blocking mitochondrial dynamics in GBM-astrocyte co-cultures, or specifically in donor astrocytes, impacts the acquisition of exogenous mitochondria by GBM cells. Thus, we provide evidence for the regulation of mitochondrial transfer by key players in fusion and fission, representing a novel therapeutic approach to curtail the metabolic reconfiguration that promotes GBM progression and resistance.

Age-Related Decline in Hippocampal Microglial Dynamics: Implications for Neurodegeneration and Disease Progression

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Alterations in microglia are implicated, among others, in the pathogenesis of age-related diseases, including Alzheimer's disease (AD). These changes are well characterized at the molecular and morphological levels, but not extensively at the dynamic level. In the mouse retina, microglial dynamics are known to change in an age-dependent fashion (Damani et al., 2011), with microglia maintaining a 'steady-state' but with quantitatively diminished dynamics. Similarly, studies in the mouse brain using in vivo cranial windows (Hefendehl et al., 2014) have shown age-related reductions in microglial process motility. To explore these dynamic changes further, we used acute brain slices. Our results demonstrate that the observed age-dependent decline in microglial dynamics, in the hippocampus, aligns with previously reported trends, confirming that aging significantly reduces the motility and process extension-retraction activity of microglia. These findings underscore the importance of microglial dynamic alterations in the aging process and contribute to the understanding of microglial behavior in the context of neurodegenerative disease progression.

Alterations in plasticity and inhibitory activity in medial prefrontal cortex a double-hit model of schizophrenia in rat

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Schizophrenia is a psychiatric disorder of complex etiology characterized by cognitive, psychotic and affective symptoms. This disease presents alterations of the excitatory/inhibitory balance at the medial prefrontal cortex (mPFC), a brain region that plays a crucial role in cognitive functions such as decision making, working memory and impulse control.

We have used a double-hit model of schizophrenia (MK-801 injection at P7 and social isolation) in rats of both sexes to evaluate the structural and functional state of the mPFC related to the behavioral phenotype.

In the sucrose preference test, male rats show a decrease in sucrose preference, an indicator of anhedonia-like behavior. In the open-field test, male rats exhibit an increase in distance traveled and time spent in the center of the arena, and a decreased immobile time, indicating hyperlocomotion. In female rats, we observed the same trend. A functional study of the mPFC was performed by stimulating the amygdala and the medialdorsal nucleus of the thalamus and recording at the level of the prelimbic and infralimbic regions. Pulse-pair inhibition has been observed in control animals with both amygdala and thalamus stimulation, whereas in schizophrenia model rats, pulse-pair facilitation occurs in both cases. At the histological level, we have observed an increase of parvaalbumin interneurons in female rats and a decrease in male rats in the infralimbic region. We have also studied Otx2, an indicator of neuronal plasticity, finding an increased expression in both the prelimbic and infralimbic regions in males and females.

As a conclusion, a disinhibition of medial prefrontal cortex activity is occurring in our schizophrenia model mediated by PV cells and other plasticity markers, provoking altered behavior in males and females.

Alterations in the activity and connectivity of the medial prefrontal cortex after overexpression of the mutant A53T human α-synuclein in the serotonergic connectome in female mice. Involvement in anxiety disorders in PD

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Parkinson's disease (PD) is a motor disorder, although it is now recognized that non-motor symptoms can precede PD motor manifestations and are an important component at all disease stages. Among these, depression and anxiety are the most prevalent symptoms and cause a higher symptom burden in women than in men. Abnormalities in the serotonergic (5-HT) system are often associated with mood disorders, and aggregates of α-synuclein (α-Syn) have been reported in 5-HT raphe nuclei of patients with PD and depression. To investigate the role of the ventromedial prefrontal cortex (vmPFC)-dorsal raphe nucleus (DR) circuit in depressive/anxiety disorders in PD, we used a female mouse model of α-synucleinopathy in the 5-HT system, based on AAV1/2-induced overexpression of A53T mutant human-α-synuclein (h-α-Syn), in raphe nuclei. The anxious/depressive phenotype was examined using several behavioral tests. Neuronal activity of infralimbic cortex (IL) and prelimbic cortex (PL) in awake mice was assessed by single-unit recordings and LFPs, using a multichannel probe and a virtual reality corridor. Functional and structural connectivity of interconnected brain areas was analyzed using functional magnetic resonance imaging (fMRI). Synaptic markers and h-α-Syn expression were also examined by western blot. Results showed that female mice overexpressing h-α-Syn in 5-HT neurons develop an anxiety-like phenotype. In parallel, h-α-Syn overexpression in the 5-HT system changed the firing rates of pyramidal and GABAergic neurons (somatostatin and parvalbumin interneurons) in the PL and IL, as well as brain connectivity. At the same time, changes in synaptic markers (SV2A, PSD95, synaptophysin) were found in PFC. Ketamine treatment reversed some of these alterations and the anxiety-related phenotype. Overall, this study suggests that overexpression of h-α-Syn in 5-HT neurons alters the functionality and connectivity of the vmPFC-DR circuit, resulting in an anxious phenotype in female mice

Alterations of the spleen immune system during neuronal degeneration in PCD mice

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The immune and the central nervous systems maintain a complex relationship that may be altered in neurodegenerative processes. The spleen plays a fundamental role in the systemic immune response by filtering the blood, modulating lymphocyte activation and serving as a reservoir of immune cells. Immune cell alteration in the spleen may reflect or influence the progression of some neurodegenerative diseases.
The present study aims at analysing the immunological changes associated with neuronal degeneration in the Purkinje Cell Degeneration (PCD) murine model. This animal suffers the death of both Purkinje and mitral cells in the cerebellum and the olfactory bulb (OB) respectively, the former degeneration being more rapid and aggressive. Through flow cytometric analysis of the spleen, we evaluated changes in myeloid and lymphoid cell populations along both degenerations, also including pre- and post-degenerative stages. We have focused on markers of functional maturation and antigen presentation.
Our preliminary results suggest that alterations in spleen immune cells during the degeneration of the OB in PCD mice are not as numerous as those previously described during the cerebellar one, characterized by a lower proportion of certain myeloid populations in the spleen, together with reduced markers of antigenic presentation and functional maturity.
Our laboratory's cumulative findings indicate that neurodegeneration in the PCD model impacts the immune system in a specific manner, with differential effects depending on the affected brain region. The lower immune response in the OB, compared to the cerebellum, could be attributed to the lesser severity of the degenerative process in this region. A better understanding of these interactions could offer novel insights into the development of therapeutic strategies to regulate the immune response in these disorders.

Funding: MICINN (PID2022-140456NB-I00), JCyL-FEDER (SA112P24), ISCIII (PI24/00447, CB22/05/00016). EMB-A holds a JCyL postdoctoral contract (SA112P24) and IP-L a MICIU predoctoral fellowship (FPU22/00693).

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Altered emotional behavior and synaptic plasticity in a Parkinson's disease model with α-Synuclein overexpression in the mPFC of female mice

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Neuropsychiatric disorders, including anxiety and depression, affect around 50% of individuals with Parkinson's disease (PD), often-preceding motor symptoms. Neuropathological changes in ventromedial prefrontal cortex (vmPFC), crucial for emotion regulation, memory, and decision-making, are strongly associated with mood disorders. Neuroimaging studies reveal vmPFC network changes in depressive PD patients that differ from those of patients with PD alone. Although the etiology of PD remains unclear, the accumulation of α-synuclein (α-Syn) protein in several neurotransmitter systems and brain areas plays a crucial role in the disease process. Indeed, neuropathologic studies show that almost 50% of PD patients do not follow a caudal-rostral progression of α-synucleinopathy, supporting the hypothesis that pathogenic α-Syn originates in corticolimbic areas before spreading throughout the body. We investigated whether overexpression of α-Syn in mouse mPFC induces structural and functional changes in brain circuits involved in emotional regulation. We developed a female PD-like mouse model based on AAV2/5-induced bilateral overexpression of wild-type human-α-Syn (h-α-Syn) in mPFC (prelimbic and infralimbic cortices). Behavioral studies were performed 4, 8, and 24 weeks after the AAV2/5 vector infusion to assess motor function, anxiety and depressive-like behaviors, and cognitive performance. H-α-Syn and phospho-α-Syn spreading, and their co-localization with different brain cell types were assessed by immunofluorescence. Dendritic spine density and morphology were examined using Golgi-Cox staining. Female mice overexpressing h-α-Syn in mPFC presented an anxious-like phenotype and spatial memory alterations compared to control groups. In addition, they showed time-dependent accumulation of h-α-syn and phospho-α-syn in mPFC and projection brain areas. Preliminary morphological analysis suggests changes in dendritic spine profiles in mice overexpressing h-α-Syn compared to controls. Our findings suggest that α-Syn overexpression in the mPFC of female mice leads to significant behavioral and structural synaptic alterations in a PD mouse model, providing insight into the role of mPFC in the neuropsychiatric symptoms of PD.

Altered excitability of developing CA1 pyramidal neurons in Fragile X Syndrome

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Fragile X Syndrome (FXS) is the most common inherited autistic spectrum disorder and is accompanied by serious intellectual disability and multiple behavioral alterations, such as hyperactivity, anxiety, social impairments as well as learning and memory deficits. The latter have been linked to alterations in excitability and synaptic plasticity mechanisms in the hippocampus. Here, whole-cell patch-clamp in current-clamp configuratiogmailn was used to study the action potential features, after-potentials and firing properties of CA1 pyramidal neurons from animals of 15-20 days old, in a mice model for FXS (Fmr1-KO). Fmr1-KO mice showed action potentials with increased width and amplitude. Interestingly, these mice presented after-depolarization potential instead of fast after-hyperpolarization potential that evokes a weak burst firing-patten. Finally, these neurons presented a leftward-shift of the input-output curve and an increased maximum firing frequency. These results are aligned with other works performed in adult Fmr1-KO mice and show a general increment in CA1 pyramidal neurons excitability.

Amyloid-β oligomers induce early astro- and microgliosis in male and female mice

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Under physiological conditions, microglia plays a fundamental role in brain homeostasis by participating in immune surveillance, while astrocytes contribute to maintaining neuronal function and synaptic activity. A key pathological feature of Alzheimer's disease (AD) is neuroinflammation, characterized by aberrant activation of these glial cells in response to the accumulation of amyloid-β (Aβ) peptide and hyperphosphorylated tau, particularly in the hippocampus. This leads to an early and chronic pro-inflammatory state, associated with excessive release of pro-inflammatory cytokines, oxidative stress, and synaptic dysfunction, all of which contribute to neuronal damage and cognitive decline. Despite increasing evidence supporting the role of neuroinflammation in AD, questions remain regarding how glial activation differs by sex and across disease progression.
In this study, we evaluated neuroinflammation in both male and female mice in the early stages of AD by using a model of acute hippocampal amyloidosis induced by intracerebroventricular (icv.) injection of soluble amyloid-β peptide oligomers (oAβ), involved in the early deficits observed in AD. Glial reactivity was analyzed in the dorsal hippocampus 24 hours post-icv. injection of oAβ by assessing astrocyte and microglial markers for GFAP and Iba-1 respectively, using immunohistochemical optical density analysis.
Preliminary results show significant alterations in GFAP and Iba-1 levels 24 hours post-icv. injection, without differences between sexes.
These findings suggest that in early stages of AD, oAβ triggers glial reactivity in both male and female mice which may underlie synaptic plasticity and memory alterations in this model. Moreover, these results point out glial modulation as a potential therapeutic target for AD in its earliest stages, opening opportunities for interventions aimed at restoring neuronal homeostasis and cognitive function in both sexes.
Funding: PID2020-115823-GBI00 (MCIN/AEI/10.13039/501100011033/ERDF), SBPLY/21/180501/000150 (JCCM/ERDF) and 2022-GRIN-34354 (UCLM) both to LJ-D and JDN-L. RDM held a predoctoral FPI contract by UCLM (2023-UNIVERS-11983).

Analysis of behavioral and senescence-related markers following a chronic senolytic treatment in 5xFAD mice

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, with aging being the biggest risk factor. In this context, cellular senescence, a stable and terminal state of growth arrest, plays a key role. Senescent cells accumulate in aging tissues, including the brain, and release pro-inflammatory cytokines and proteases, potentially contributing to AD pathophysiology and acting as a link between aging and AD. Thus, modulating cellular senescence could be a promising target for the treatment of AD-linked dementia.

In this study, we analyzed the effect of administering a chronic senolytic compound to 5-month-old 5xFAD mice. The 5xFAD mouse model is a transgenic model used to study AD, carrying five familial mutations, three in the amyloid precursor protein (APP) gene and two in the presenilin-1 (PS1) gene. Following the senolytic treatment, we performed a battery of behavioral tests to assess cognitive function with these mice and age-matched controls, including tasks for evaluating anxiety, working memory, as well as recognition and spatial memory. Additionally, senescence and neuroinflammation markers, as well as potential gliosis, were studied using immunohistochemistry and colorimetric imaging methodology. Given the observed overlap between amyloid plaques and senescence-associated β-galactosidase (SA β-Gal) activity, a key marker of cellular senescence, we hypothesize that the senolytic treatment could have a therapeutic effect in behavior and neuroinflammation parameters.

Overall, the senolytic treatment might improve some of the main pathological hallmarks of AD, including a reduction in Aβ plaques in key brain areas for learning and memory processes. These findings suggest that senolytic therapies hold promise for alleviating cognitive decline and reducing the excessive presence of senescent cells in brain tissue affected by AD pathology.

Anti-seizure Gene Therapy for Focal Cortical Dysplasia type II

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The Focal Cortical Dysplasias (FCDs) are a group of malformations of cortical development, which are frequently associated with drug-resistant epilepsy in children. FCD type II is the commonest pathology found at epilepsy surgery in children. It is typically caused by somatic mutations resulting in the mammalian Target Of Rapamycin Complex 1 (mTORC1) hyperactivation. Epilepsy surgery is not always effective and is often precluded by proximity to eloquent brain regions. Gene therapy is thus currently the most promising candidate replacement for surgical treatment of FCD.
We tested a gene therapy approach based on viral expression of a modified KCNA1 gene (which encodes the potassium channel Kv1.1). We chose Kv1.1 as a therapeutic protein not only because it attenuates neuronal excitability, but also because mTOR hyperactivation is associated with downregulation of KCNA1 expression.
The therapy tested in a mouse model of FCD type II generated by in-utero electroporation of neuronal progenitors with a constitutively active RHEB plasmid, an activator of mTORC1. A battery of molecular biology and behavioural tests confirmed that the model recapitulates the pathology seen in humans, including cognitive comorbidities. Continuous EEG recordings from mice before and after local injection of AAV9-EKC, showed a 64% decrease in seizure frequency over 4 weeks (n=13 mice), which was not seen in mice injected with a control AAV encoding a fluorescent reporter alone (n=11 mice). We observed no worsening of performance in tests sensitive to frontal lobe function, including spontaneous alternation in T-maze and olfactory habituation and discrimination.
In conclusion, EKC gene therapy is a promising genetic approach for the treatment of FCD type II.

APOE polymorphism affects the morphology and functionality of human iPSC-derived astrocytes in Alzheimer's disease: Impact on hippocampal neurons

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Alzheimer's disease (AD) is the leading cause of dementia in the aging population, with the ε4 allele of apolipoprotein E (APOE) being the strongest genetic risk factor. Astrocytes are pivotal in mediating key processes during the progression of AD, including the clearance of amyloid-beta (Aβ) aggregates and the inflammatory response. Nevertheless, the impact of different APOE alleles on astrocyte development, maturation, and function remains to be fully elucidated. In this study, we obtained induced pluripotent stem cells (iPSCs) from fibroblasts of AD patients carrying ε3 and ε4 alleles (in homozygosis) and from healthy individuals. We also used gene-edited iPSC lines homozygous for the main APOE variants and an APOE knock-out line. Human astrocytes were generated by establishing a differentiation protocol by adding small molecules and growth factors. The expression of characteristic markers and APOE were then analysed to confirm the astrocytic phenotype. In addition, human astrocytes exhibited calcium wave production and glutamate uptake capacity. They also responded to an inflammatory stimulus by changing their morphology and increasing the expression levels and release of pro-inflammatory cytokines such as IL-6. The present findings suggest that APOE polymorphism may influence the basal and inflammatory state of astrocytes, as well as their ability to respond to stimuli by acquiring different morphologies, which could potentially impact hippocampal neurons; the latest is being studied through astrocyte-neuron co-cultures. Furthermore, the ε4 allele may modify Aβ uptake/degradation capacity by astrocytes and its distribution within the cell. The present findings underscore the significance of APOE polymorphism in the morphological and functional profile of astrocytes and their potential correlation with the risk of developing Alzheimer's disease (AD). Additionally, astrocyte-neuron co-culture experiments may provide a means to elucidate the influence that the APOE polymorphic variant-associated phenotype in astrocytes may have on surrounding hippocampal neurons

Aquaporins, Brain Barriers, and Hydrocephalus: The Crucial Role of p73

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P73 gene, which belongs to P53 gene family, plays a critical role in the central nervous system (CNS) development. Transgenic KOp73 mice exhibit a characteristic phenotype with multiple alterations, including hydrocephalus. The aim of this study was to determine the underlying processes of hydrocephalus in these mice.
To this end, we analyzed the expression of aquaporins 1 (AQP1) and 4 (AQP4) in the blood-brain barrier (BBB), blood-cerebrospinal fluid barrier (BCSFB), and the cerebrospinal fluid-brain interface (CSF-BI) (ependymal layer) using immunoperoxidase and immunofluorescence techniques.
Our results showed: an increase in AQP1 expression in the choroid plexus (BCSFB) at all life stages in KOp73 mice, even before the onset of hydrocephalus; increased AQP4 expression in the BBB and decreased AQP4 expression in the CSF-BI after ependymal loss. These findings allowed us to identify two key factors contributing to hydrocephalus:

1. Overexpression of AQP1 and the consequent increase in CSF production.
2. Ependymal loss, leading to CSF accumulation in the ventricles.

As a compensatory mechanism, we only identified AQP4 overexpression in the glia limitans and perivascular spaces of the brain parenchyma. However, this compensatory response is insufficient to fully reabsorb the excess fluid.
Overall, these findings suggest that P73 could be an important gene for the proper functioning of brain barriers and, ultimately, for CSF homeostasis.

Astrocyte-derived mitochondria, a potential threat for striatal neurons in Huntington's disease

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Mitochondrial dysfunction plays a central role in the pathogenesis of several neurodegenerative diseases. Particularly, in Huntington's disease (HD) -an inherited neurodegenerative disorder characterized by psychiatric, cognitive and motor impairments -mitochondrial abnormalities are prominent. These mitochondrial dysfunctions, along with other cellular alterations, are most notably observed in neurons within the striatum, the brain region primarily affected in HD.

Under normal physiological conditions, astrocytes and neurons engage in a process known as transmitophagy, where mitochondria are exchanged between these cell types. This process is beneficial for neurons in terms of improving neuronal metabolism. However, the role of transmitophagy in neurodegenerative diseases remains largely unexplored. Our group is focused on describing the consequences of transmitophagy from striatal astrocytes to striatal neurons in the R6/1 mouse model of HD. To investigate this, we first characterized mitochondrial function and mitochondrial network in primary cultures of R6/1 astrocytes and analyzed the impact of astrocytic mitochondrial uptake on cultured striatal neurons. Our results demonstrate that mitochondria in R6/1 astrocytes exhibit an abnormally accelerated respiration rate and increased fission, as well as a decreased mitophagy clearance. Interestingly, astrocytes release mitochondria into the extracellular medium, which can be internalized by striatal neurons. Upon internalization of R6/1 astrocytic mitochondria, striatal neurons exhibit increased reactive oxygen species (ROS) levels, and defective neuronal branching. These results suggest that, in the context of HD, transmitophagy may exacerbate neurodegeneration, highlighting its potential as a therapeutic target in future HD treatments.

Axotomy induces upregulation of NKCC1 cotransporter in rat extraocular motoneurons that is prevented by VEGF, but not BDNF, administration

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The sodium-potassium-chloride cotransporter NKCC1 is the main transporter responsible for the influx of Cl- ions into neurons. It plays a crucial role in modulating the activity of the inhibitory neurotransmitters (GABA and glycine), since high levels of NKCC1 are associated with a high Cl- intracellular concentration. This increased Cl- concentration leads to the efflux of this anion when inhibitory neurotransmitters bind to their receptors, thus resulting in neuronal depolarization. In addition, NKCC1 also transports water molecules into the cell along with ions, regulating cell volume. High levels of NKCC1 have been linked to neurological and psychiatric disorders characterized by hyperexcitability.
However, little is known about the response of NKCC1 to axotomy and its regulation by neurotrophic factors. In the present work, we have induced the axotomy of extraocular motoneurons and examined the levels of this cotransporter in the extraocular motor nuclei (oculomotor, trochlear and abducens) after vehicle or neurotrophic factor administration (VEGF or BDNF). Under general anesthesia, unilateral enucleation was performed in adult female rats, and then a Gelfoam soaked in either VEGF or BDNF (1 µg/20 µl in PBS-0.1% BSA) or in the vehicle was implanted in the orbit and eyelids were sutured. Fifteen days later, animals were intracardially fixed, and their brainstem sectioned into 30-µm-thickness coronal sections. Double immunofluorescence of ChAT (to identify motoneurons) and NKCC1 was then performed using confocal microscopy.
Our results demonstrated that axotomy induced an upregulation in NKCC1 levels in extraocular motoneurons, which was prevented by VEGF, but not by BDNF, administration, as this neurotrophin maintained the high NKCC1 levels observed in axotomy. Likewise, axotomized motoneurons also showed increased cell size, which VEGF prevented, but not BDNF. These findings point to NKCC1 and VEGF as potential therapeutic targets for the treatment of neurological damage or diseases characterized by hyperexcitability.

Blood-derived macrophages infiltrating the injured brain drive lineage plasticity in glia

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Several studies have demonstrated that mouse and human adult cortical astrocytes can dedifferentiate and form multipotent neurospheres following brain injuries that disrupt tissue compartmentalization. Consistent with this acquisition of lineage plasticity after lesion, glia-to-neuron reprogramming is more effective in injured brains than in uninjured ones. In this context, CSF containing galectin-3, mainly originating from the choroid plexus and leaking into the brain parenchyma after invasive injuries, plays a critical role in endowing astrocytes with multipotency. However, the precise mechanisms, the origin of galectin-3 within the CSF, and the involvement of other extraparenchymal factors remain unclear. To address these questions, we developed a reactive culture (RC) model composed of cells from the injured cortex, including reactive astrocytes, microglia, infiltrated macrophages, pericytes, and NG2+ cells, and tested whether they induce the dedifferentiation of co-cultured non-reactive adult glia. We found that reactive cells promote neurosphere formation from non-injured cortical and substantia nigra glia. Next, we asked whether macrophages alone could reproduce this effect, as they are the only extra parenchymal cells in the RC model and typically infiltrate from the bloodstream in invasive and traumatic lesions. Indeed, bone marrow-derived macrophages not only induced neurosphere formation in glial cells from the uninjured adult brain but also enhanced neuronal reprogramming efficiency in postnatal astrocytes transfected with pro-neuronal factors. Finally, to identify which macrophage-secreted molecules drive these effects, we performed a proteomic analysis of conditioned medium from macrophages and RCs, comparing their secretome with that of astrocytes and fibroblasts, which do not induce neurosphere formation. This analysis revealed abundant macrophage-secreted factors, including galectin-3, with key roles in neural stem cell maintenance and multipotency. In conclusion, our study demonstrates that immune cells drive lineage plasticity in the injured brain, representing a crucial step in tissue regeneration and offering a promising perspective for neuronal reprogramming strategies.

Cannabidiol Administration during Pregnancy and Lactation in a Context of Maternal Alcohol Exposure Alters Glutamatergic System and Cell Proliferation in Offspring Hippocampus

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Excessive alcohol consumption during pregnancy causes neurodevelopmental disorders with serious consequences on cognition. The role of CBD, a phytocannabinoid with neuroprotective effects, in neurogenesis in a context of alcohol exposure is still largely unknown. The present study examines the effect of maternal exposure of 20 % ethanol (ad libitum orally during 2 or 4 hours/day, 4 days/week) in offspring and its response to a prior subcutaneous injection of CBD (20 mg/kg) for 6 weeks during pregnancy and lactation. This study assessed gene expression related to glutamatergic and endocannabinoid systems —key components of synaptic transmission modulation—, beside cell proliferation in the subgranular zone (SGZ), in the hippocampus of mouse offspring of both sexes at postnatal day (PND) 21. Novel object recognition (NOR) test was performed in mouse offspring of both sexes at PND60. Our findings showed that CBD decreased NOR preference ratio, an effect that was accompanied by reduced SGZ cell proliferation, in male offspring born to control dams, but not after maternal alcohol exposure. CBD also had an opposing effect on mRNA expression of NMDA receptor subunits 1 (Grin1) and 2b (Grin2b): both increased their levels in the hippocampus of offspring from dams who drank water and decreased after maternal alcohol exposure. Moreover, mRNA levels of Grin2a and the AMPA receptor subunit Gria2 were increased with CBD and maternal alcohol exposure, while the phytocannabinoid reduced glutaminase 2 (Gls2) values. Furthermore, CBD had a strong effect on decreasing the expression of cannabinoid receptor 2 (Cnr2) and protein tyrosine phosphatase non-receptor type 22 (Ptpn22). In summary, our study suggests that CBD alters glutamatergic receptor expression, possibly through its action on cannabinoid receptor 2, impacting SGZ cell proliferation and memory.

Cannabinoid CB1 receptorsmodulate oligodendrocyte maturation during remyelination

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Oligodendrocytes, the myelin-producing cells of the central nervous system, arise from oligodendrocyte progenitor cells (OPCs) both during development and during the process of remyelination. Their differentiation is a complex, multi-step process regulated by various extracellular signals. However, the full spectrum of signaling pathways governing oligodendrocyte maturation remains incompletely understood. Cannabinoids have been identified as key modulators of oligodendrocyte development, influencing both myelin formation and repair. Specifically, CB1 cannabinoid receptors play a crucial role in the early stages of OPC differentiation in both developmental and regenerative contexts. However, their function in the final stages of oligodendrocyte maturation is less clear. Using conditional genetic mouse models, we show that CB1 receptor depletion in oligodendrocytes disrupts late-stage maturation during myelin repair by affecting mTORC1 and RhoA/ROCK signaling pathways. In a cuprizone-induced demyelination model, loss of CB1 receptors in oligodendrocytes impairs CNS remyelination, exacerbates gliosis and axonal damage, and prevents motor function recovery. These findings reinforce previous pharmacological evidence highlighting the essential role of CB1 receptors in oligodendrocyte maturation during myelin repair and suggest that cannabinoids may hold therapeutic potential for enhancing myelin regeneration.

CB1 receptors in NG2 cells mediate cannabinoid-evoked functional myelin regeneration

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Myelin integrity and function are often compromised in various neurological disorders,severely affecting neuronal health. Cannabinoid compounds have been shown to influence oligodendrocyte regeneration, to promote CNS remyelination, and to enhance functional recovery in several demyelination models. However, the direct role of cannabinoids in NG2 cells, which contribute to remyelination, remains unclear. In this study, we employed a genetic model targeting oligodendrocyte precursor cells (OPCs) to investigate the function of CB1 receptors in oligodendrocyte differentiation and remyelination using the cuprizone demyelination model. Our findings indicate that CB1 receptors loss in OPCs impairs CNS remyelination, worsens axonal damage, and prevents motor and cognitive recovery. Notably, pharmacological inhibition of the RhoA/ROCK pathway counteracted these detrimental effects, restoring oligodendrogenesis and functional remyelination. These results point to CB1 receptors as key regulators of NG2 cell differentiation in response to demyelination and highlight the potential of cannabinoid-based therapies for enhancing remyelination in neurological diseases.

CD200-based cell sorting drives a safer and more reproducible cell therapy for Huntington's Disease

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Cell replacement therapy (CRT) holds promise to restore the degenerated brain tissue in Huntington's Disease (HD) by replenishing lost striatal projection neurons (SPNs). However, clinical translation is hindered by the heterogeneity in cell compositions produced through striatal differentiation protocols, compromising both safety and reproducibility. Demonstrating the absence of uncontrolled cell proliferation is thus essential. This study aims to develop a novel CRT strategy for HD by selecting specified striatal post-mitotic neuroblasts (NBs) while excluding proliferative cell types.
To achieve this, we established an immunomagnetic sorting pipeline to select NBs derived from human pluripotent stem cells (hPSCs). We characterized the identity of the sorted cell subpopulations and evaluated their safety and fate following transplantation into the striatum of immunosuppressed adult mice.
We identified CD200 as a marker for post-mitotic NBs, enabling consistent, high-yield enrichment of CD200-high NBs. Sorted cells exhibited hallmark characteristics of SPN-committed NBs and differentiated into SPN-like neurons in vitro. Following transplantation, CD200-high NBs retained the expression of striatal markers and continued to differentiate for at least one month, underscoring their regenerative potential for HD. Notably, CD200-based selection provided a key safety advantage by eliminating neural rosette-like structures, which appeared frequently in unsorted cell grafts. The proposed strategy significantly reduced the percentage of proliferative cells in the grafts, minimizing the risk of graft overgrowth and tumor formation in vivo.
In conclusion, our findings highlight the translational value of CD200-based cell sorting in meeting quality and safety requirements for future CRT in HD.

Cell-type specific profiling of human entorhinal cortex at the onset of Alzheimer's disease neuropathology

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Neurons located in layer II of the entorhinal cortex (ECII) are the primary site of pathological tau accumulation and neurodegeneration at preclinical stages of Alzheimer's disease (AD). Exploring the alterations that underlie the early degeneration of these cells is essential to develop therapies that curb the disease before symptom onset. Here we performed cell-type specific profiling of human EC at the onset of AD neuropathology. We identify an early response to amyloid pathology by microglia and oligodendrocytes. Importantly, we provide the first insight into neuronal alterations that coincide with incipient tau pathology: the signaling pathway for Reelin, recently shown to be a major AD resilience gene is dysregulated in ECII neurons, while the secreted synaptic organizer molecules NPTX2 and CBLN4, emerging AD biomarkers, are downregulated in surrounding neurons. By uncovering the complex multicellular landscape of EC at these early AD stages, this study paves the way for detailed characterization of the mechanisms governing NFT formation and opens long-needed novel therapeutic avenues.

Cellular distribution of pyruvate kinase M2 after spinal cord injury: Microglia and macrophages

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Pyruvate kinases are key enzymes of glycolytic metabolism of neurons and glia. One of them, pyruvate kinase M2 (PKM2) can also act as a transcriptional regulator and in this function has been implicated in several neurodegenerative diseases. Since nuclear translocation of PKM2 induces the expression of inflammatory genes in macrophages [Palsson-Mcdermott et al. Cell Metab 21 (2015): 65.89], we hypothesized that this mechanism contributes to the neurodegenerative processes after spinal cord injury (SCI). Using a rat model of contusion SCI, quantitative RT-PCR showed a strong upregulation of PKM2 transcripts close to the lesion center. To understand the functional implication of this observation, we studied PKM2 antigen expression in microglia and macrophages. Spinal cord injury caused a significant increase of PKM2 immunoreactivity in microglia, correlating with proximity to the lesion site. Macrophages were strongly PKM2 positive and displayed a marked accumulation of PKM2 in the lesion core, suggesting its involvement in the inflammatory and phagocytosis response. An analysis of the intracellular distribution revealed that microglia in various states of activation, including the phagocytic phenotype displayed PKM2 in both the cytosol and nucleus. The traumatic lesion did not induce a significant nuclear translocation of PKM2 but caused an increase of protein expression in both compartments. Our data stress the involvement of PKM2 after SCI and implicate its metabolic function as well as a transcriptional regulator of inflammation.

Cellular distribution of pyruvate kinase M2 after spinal cord injury: Neurons, glia and apoptosis

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Traumatic spinal cord injury (SCI) causes substantial metabolic changes associated with the loss of tissue homeostasis. Pyruvate kinases are key enzymes of glycolytic metabolism of neurons and glia. One of them, pyruvate kinase M2 (PKM2) can also act as a transcriptional regulator and in this function has been implicated in several neurodegenerative diseases. Two previous studies found SCI caused an increase of PKM2 expression in astrocytes [Zhang et al. Cell Cycle 14 (2015): 2609] and microglia [Romero-Ramírez et al. Sci. Rep. 12 (2022): 1459].
To understand the role of PKM2 in other cell types after trauma we used a rat model of contusion SCI and studied PKM2 antigen expression in the tissue. In addition, we used TUNEL staining to investigate whether levels of PKM2 correlate with apoptosis after SCI.
Spinal cord injury caused a significant increase of PKM2 immunoreactivity in astrocytes and motor neurons but not in interneurons and oligodendrocytes. The expression in astrocytes increased notably in the chronic phase surrounding the lesion site, consistent with formation of the glial scar. The analysis of the intracellular distribution revealed that astrocytes and oligodendrocytes displayed PKM2 in both the cytosol and nucleus, whereas PKM2 positive neurons were devoid of nuclear staining. The SCI did not induce a significant nuclear translocation of PKM2 in any of these cell types. Despite rising levels of PKM2 and TUNEL staining after SCI, we found no correlation between PKM2 and apoptosis. Our data indicate that the activity of PKM2 is differently regulated in neurons and glial cell types, where it fulfills different physiological functions.

Changes in the excitation-inhibition equilibrium of Dentate Gyrus Granule Cells (DGGC) in an adult animal model of autism induced by VPA

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Autism is the most common Autism Spectrum Disorder (ASD) affecting approximately 1% of the world population. The etiology of this disorder is still unknown, however, environmental factors such as prenatal exposure to valproic acid (VPA) are involved in the ASD generation. ASD is characterized by deficits in social interaction, language trouble, deficient communication, and stereotyped repetitive behaviors. Magnetic Resonance Imaging have shown structural changes in the hippocampus and amygdala from subjects with autism, which correlates with impaired memory, learning and social management of emotions. Studies in humans and animals have shown changes of neurotransmitters in areas such as the cortex and the hippocampus. About 50% of autism'patients have epilepsy in adult stages. Evidence showed that both disorders share mechanisms, such as alteration in the excitation-inhibition equilibrium, changes in the morphology of structures if the temporal lobe and changes in brain electrical activity. The main evidence has identified changes in the level of expression of GABA receptor subunits and chloride co-transporters. This research focuses on studying the glutamatergic and GABAergic changes in the dentate gyrus in an autism animal model and how these increase the probability of generating seizures. Through the generation of behavioral tests of social interaction and anxiety, the animal model induced by VPA was validated. On the other hand, extracellular field recordings in VPA animals identified GABAergic and glutamatergic alterations in the dentate gyrus, where an increase in excitation in VPA animals was observed. This laid the foundations for understanding the mechanisms by which the comorbidity of autism and epilepsy in humans is generated and suggest possible new mechanisms to explain the etiology of the disease and possible new treatments.

Characterization of the effect of remote ischemic conditioning on peripheral organs post-ischemic stroke: impact on tissue inflammation

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Ischemic stroke represents a major global health problem, being one of the leading causes of mortality and morbidity. Currently, two therapeutic strategies are available, both focused on cerebral revascularization. However, recent studies have shown that the effects of strokes are not limited to the brain, but also trigger systemic processes such as inflammation. Remote ischemic conditioning (RIC) is an emerging strategy based on ischemia-reperfusion cycles applied to the limbs, which has been shown to reduce cerebral infarct volume and to have neuroprotective effects. The present study aims to explore the effects of RIC on systemic inflammation in vital peripheral organs following stroke.

We evaluated endothelial activation and the expression of adhesion molecules using western blot, and the infiltration of monocytes and lymphocytes with immunofluorescence. Both approaches focused in the heart, liver, kidney, and intestine of adult CD1-mice subjected to distal middle cerebral artery occlusion for 1 hour. RIC consisted of 3 cycles of 5 minutes each applied during (Per-RIC) or immediately after occlusion (post-RIC). Additionally, alterations in gut microbiota were analysed by 16S RNA sequencing.

The results obtained suggest that RIC differentially modulates systemic inflammatory responses. Per-RIC increases the expression of molecules such as AIF and Annexin V, associated with endothelial dysfunction. It also modifies immune cell infiltration in the studied organs. In contrast, post-RIC does not induce endothelial damage, although it also modifies leukocyte infiltration. Additionally, subtle changes in the composition of the gut microbiota were observed in post-RIC.

These results suggest that post-RIC may have effects beyond the brain, modulating the post-stroke systemic inflammatory response in peripheral organs and potentially modulating the gut-brain axis. These findings help establish the potential of RIC as a therapeutic strategy for acute stroke and post-stroke systemic effects, in addition to suggest that the timing of RIC application is key to its effectiveness.

Chronic treatment with adenosine A1 antagonist promotes neurogenesis and improves outcome after cerebral ischemia

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Adenosine A1 receptors (A1ARs) are promising targets for stroke treatment. However, their role in proliferation and differentiation of newborn neurons remains largely unexplored. Given the current lack of effective treatments, bridging this gap is critical for developing novel neuroprotective strategies to manage chronic stroke. In this study, we investigated the impact of chronic treatment with the A1ARs antagonist DPCPX on neurogenesis following transient middle cerebral artery occlusion (MCAO) in rats and mice, using positron emission tomography (PET) with [^{18}F]FLT and immunohistochemistry (IHC), respectively. In addition, we assessed the therapeutic properties of DPCPX on stroke recovery with a comprehensive battery of neurological and behavioral tests. Our findings demonstrate that blocking A1ARs signaling with DPCPX improved outcomes after brain ischemia in mice. PET imaging with [^{18}F]FLT revealed increased cellular proliferation following DPCPX treatment in the subventricular zone (SVZ) at day 8 post-ischemia in rats, a result further supported by IHC in SVZ of ischemic animals. Furthermore, DPCPX enhanced the production and integration of newborn neurons while reducing astrocytic differentiation in the ischemic areas. Finally, behavioral tests showed that chronic treatment with DPCPX ameliorated motor and memory deficits after brain ischemia. Taken together, our results provide novel and compelling evidence of the therapeutic potential of the A1AR antagonist DPCPX for ischemic stroke recovery.

Clozapine Induces Perineuronal Net Remodelling in a Developmental Mouse Model of Schizophrenia

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Schizophrenia (SCZ) is a multifaceted neurodevelopmental disorder characterized by widespread dysregulation of extracellular matrix (ECM) components, particularly perineuronal nets (PNNs), which are crucial for synaptic stability and cognitive function. Alterations in PNNs, especially in the prefrontal cortex (PFC), have been linked to cognitive deficits in SCZ. While antipsychotic (AP) treatments have been suggested to influence PNNs and ECM integrity, their specific effects remain unclear. In this study, we used a neurodevelopmental mouse model of SCZ, induced by perinatal NMDA receptor hypofunction through ketamine administration, to investigate the impact of PNN alterations and their modulation by clozapine (CLZ), an atypical AP, in adulthood. We found that ketamine exposure led to increased PNN compactness, elevated VGLUT1-mediated excitatory inputs, and enhanced medial PFC activation. Treatment with CLZ mitigated these changes by restoring PNN microarchitecture in the medial PFC. Additionally, CLZ-mediated effects on PNNs were associated with improvements in cognitive flexibility and social memory, functions tightly linked to PFC-dependent processing. These findings suggest that CLZ plays a role in modulating PNN structure in the SCZ brain and underscore the need for further investigation into the impact of AP medications on PNN plasticity and ECM integrity.

Common microRNA dysregulation in major depressive disorder: cross-species insights from human postmortem brain and a stress mouse model

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Major depressive disorder (MDD) is the most prevalent mental illness worldwide and the leading cause of suicide. MicroRNAs (miRNAs) have emerged as critical regulators of gene expression, modulating numerous neurobiological functions associated with MDD. The present study examined the role of miRNAs and their regulatory pathways in the dorsolateral prefrontal cortex (dlPFC) of individuals with MDD who died by suicide (MDD/suicide) and in the medial prefrontal cortex (mPFC) and hippocampus (HPC) of a corticosterone-induced depression-like mouse model. Additionally, we further analyzed whether miRNA expression changes are sex-dependent. MiRNA sequencing was performed in the dlPFC of 17 MDD/suicide subjects (7F/10M) and 17 well-matched controls (7F/10M), as well as in the mPFC and HPC of corticosterone-treated mice (7F) and their respective controls (7F). A larger sample (66 subjects, 42M and 24F) was used for qPCR validation of miRNAseq data. mRNA targets of significantly altered miRNAs were predicted using a multi-algorithmic approach, and KEGG-based over-representation analysis was performed to identify miRNA-regulated relevant pathways. Significant global miRNA dysregulation was observed in the dlPFC of MDD/suicide subjects, with 17 miRNAs upregulated and 17 downregulated ($p \leq 0.05$). Sex-stratified analysis revealed 74 differentially expressed miRNAs in males and 54 in females, with 28 overlapping both sexes. In corticosterone-treated mice, 50 miRNAs were dysregulated in mPFC and HPC, 11 of which overlapped with human MDD/suicide profiles. KEGG analysis highlighted pathways linked to MDD pathogenesis, including neurotrophin signaling, neuroinflammation, and synaptic plasticity (long-term potentiation/depression). Overall, the present study identified common altered miRNAs associated to neuronal function and MDD pathogenesis in the dlPFC of MDD/suicide individuals and in the mPFC and HPC of a corticosterone-induced depression-like mouse model, with some showing sex-dependent differences. These findings underscore the intricate role of miRNAs in regulating critical brain functions and highlights their potential as therapeutic targets for depression.

Comparative analysis of hippocampal neurogenesis after stroke: a multicenter study on different cerebral ischaemia models

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Ischemic stroke remains one of the leading causes of death and disability worldwide. Over the past two decades, increasing evidence has suggested that hippocampal neurogenesis may contribute to the cognitive decline observed in the chronic phase of cerebral ischemia. In recent years, various experimental models have been developed to replicate different clinical aspects of ischemic stroke in laboratory settings.

As part of the Leducq Stroke IMPaCT project, this multicenter study—conducted in laboratories in Madrid, Berlin, Edinburgh, Stanford, Arizona, and New York—analyzes the hippocampal neurogenic response in adult C57BL/6J mice at different time points (72 hours, 7 days, and 9 weeks) following ischemic stroke. The study employs several ischemic models in mice, including ligation, filament occlusion, cauterization combined with hypoxia, and photothrombosis. Neurogenesis was assessed through quantitative analysis of Ki67 (cell proliferation) and DCX (neuroblasts and immature neurons) expression, alongside a morphological evaluation of the apical dendrite (AD) length and the evaluation of aberrant morphologies in DCX+ cells.

Our data demonstrate that the bilateral neurogenic response observed in the hippocampus after stroke is a conserved process across different ischemic models in mice, although its dynamics vary slightly depending on the model analyzed. Moreover, these changes are not only quantitative but also qualitative, affecting the morphology of neuroblasts after ischemia.

These findings highlight significant differences in the dynamics of hippocampal neurogenesis across experimental stroke models, underscoring the importance of model-specific approaches when investigating post-stroke neurogenesis.

Comparative analysis of NfL and GFAP levels in serum and CSF using SIMOA technology in Multiple Sclerosis patients

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system that involves demyelination and progressive neurodegeneration. Early diagnosis and disease monitoring are crucial, and biomarkers, such as light neurofilament proteins (NFL) and Glial Fibrillary Acidic Protein (GFAP), play a key role in this process. These biomarkers, present in both cerebrospinal fluid (CSF) and blood, allow for monitoring of disease activity and neurodegeneration.

The goal of this small-scale study is to compare the concentrations of NFL and GFAP in serum and CSF, aiming to demonstrate that the levels of these defined biomarkers are equal or at least correlational in both types of samples.

Traditionally, biomarkers in CSF were considered more specific for MS due to their proximity to the central nervous system, but biomarker levels in blood have also shown promising results in this area. Additionally, blood samples are easier to obtain and pose less risk to the patient.

The SIMOA (Single Molecule Array) technology is used to measure these biomarkers with high sensitivity and precision, allowing measurements at very low concentrations. SIMOA could provide more accurate results than previous methods, such as ELISA, and facilitate MS monitoring.

The possibility of monitoring and diagnosing MS through serum (not as the sole protocol, but complementary to other tests such as MRI) would facilitate sample collection, based on the comfort of both the patient and the clinician (doctor, nurse), as it is much less invasive and does not have the difficulties or complications associated with lumbar puncture. Additionally, it would enable studies on a much larger scale, impacting the diagnostic capacity and speed.

For all these reasons, comparing these biomarkers in different biological fluids could revolutionize MS monitoring, enhancing the patient experience and clinical management of the disease.

Cortico-Hippocampal Assembloids: A Novel Tool for Studying Alzheimer's Disease

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The connections established between the hippocampus and the cortex play a crucial role memory encoding, storage, and retrieval. Despite recent advances, the mechanisms underlying memory formation and the connectivity between these brain regions remain poorly understood. A major challenge in this field is the lack of suitable in vitro models to study the cortico-hippocampal pathway under physiological conditions, particularly in the context of neurodegenerative diseases such as Alzheimer's disease. To address this need, we have developed a reliable and reproducible in vitro model that replicates the development and functionality of the cortico-hippocampal pathway, with the goal of using it as a model for Alzheimer's disease. First, we derived cortical and hippocampal organoids from human induced pluripotent stem cells (hiPSCs). Characterization of these organoids through immunohistofluorescence at different time points suggests that the differentiation process recapitulates the in vivo development of both regions. Additionally, mature organoids exhibit the main cell populations and neuronal types characteristic of the cortex and hippocampus.
We analyzed neuronal activity in the organoids using calcium imaging, and the results demonstrate that the organoids display mature traits and high connectivity. To model the cortico-hippocampal pathway, we generated assembloids by combining cortical and hippocampal organoids. Interregional connectivity of the assembloids was analyzed through axonal projection studies, monosynaptic tracing, and calcium imaging. Our results reveal the formation of reciprocal projections between cortical and hippocampal organoids, leading to the establishment of functional circuits. Furthermore, to create a more physiologically relevant system that better reproduces the complex brain environment, we introduced microglial cells into the organoids. The system we have developed provides a robust and reproducible model for studying the cortico-hippocampal pathway in vitro. This platform offers a powerful tool for investigating dysfunctions in these connections in Alzheimer's disease and serves as a valuable resource for developing new therapeutic strategies.

Corticostriatal hyperactivity and synucleinopathy induce striatal astrogliosis

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Parkinson's disease is traditionally described as a bottom-up propagation of Lewy body pathology, spreading from the periphery to the central nervous system, with cortical involvement occurring only at later stages. However, growing evidence challenges this view, suggesting that cortical alterations may emerge much earlier, even during prodromal phases. Our cortical pathogenic theory (Foffani and Obeso, Neuron 2018) posits that cortical dysfunction actively contributes to disease initiation and progression through top-down mechanisms. To test this hypothesis, we developed lateralized mouse models of corticostriatal hyperactivity and synucleinopathy. We specifically injected viral vectors in the sensorimotor cortex to chronically increase corticostriatal activity with chemogenetics (DREADDs) and to overexpress wild-type or mutated A53T human alpha-synuclein in corticostriatal projections. The model of corticostriatal hyperactivity was validated with in vivo bilateral extracellular electrophysiology in anesthetized animals (n=19) and immunohistochemical staining for the EGR1, a marker of neuronal activity (n=34). The model of corticostriatal synucleinopathy was validated with immunohistochemical staining for human alpha-synuclein (n=23). We then assessed the chronic behavioural and histological impact of these corticostriatal manipulation on striatal circuits (n=105). Behavioral assessment through weekly open-field tests revealed mild hyperactivity and turning asymmetry in manipulated mice compared to sham. The manipulations did not produce a noticeable loss of striatal TH staining, a marker of nistrostriatal integrity, but they induced strong astrogliosis in the ipsilateral striatum, as assessed by a lateralized increase of GFAP expression, a marker commonly associated with nigrostriatal neuroinflammation and neurodegeneration. This lateralized increase of GFAP expression was similar in male vs. female mice, as well as with wild-type vs. mutated alpha-syuclein, and was amplified by a single dose of MPTP administered 24 h before perfusion. These findings suggest that cortical alterations can act as a top-down corticostriatal stressor, which may be relevant for the pathogenesis of Parkinson's disease and related synucleinopathies.

Cryopreservation of Human Neural Progenitor Cells for Huntington's Disease Cell Therapy

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Huntington's disease (HD) is a neurodegenerative disorder characterized by the progressive and selective loss of striatal projection neurons (SPNs). This neuronal loss makes HD an ideal candidate for cell replacement therapies (CRT). Recent advancements in differentiation protocols have enabled the efficient and scalable generation of human pluripotent stem cell-derived neural progenitor cells (hNPCs), positioning them as promising candidates for CRT.
However, translating these protocols into Good Manufacturing Practice (GMP)-compliant processes remains a major challenge. A critical step in this transition is the development of robust cryopreservation protocols for both human pluripotent stem cells (hPSCs) and hNPCs. Cryopreservation ensures rigorous in-process quality control, facilitates comprehensive safety testing, and enhances accessibility by enabling the distribution of cells to laboratories without specialized expertise or GMP-compliant facilities.
To address this challenge, we developed an optimized protocol for the maintenance, cryopreservation, and thawing of hPSCs under GMP compliance. Our protocol improves post-thaw cell recovery while preserving pluripotency, proliferation capacity, and genomic stability. Furthermore, we adapted this cryopreservation method for human neuroepithelial progenitors (hNEP) and hNPCs derived from our neuronal differentiation protocol. Although hNEPs and hNPCs exhibit lower recovery rates than hPSCs due to their greater cryopreservation sensitivity, they retain their identity and functionality after thawing. We provide a reproducible and scalable framework for transitioning from research-grade differentiation protocols to clinical applications by defining exact seeding densities and evaluating attachment rates and post-thaw viability. These advancements bring us closer to the clinical translation of cell-based therapies for HD.

Deciphering blood vessel changes in glioblastoma progression to develop targeted therapies

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Treating glioblastoma, the most common and aggressive primary brain tumour, has proven to be a true hurdle as despite combining chemo- and radiotherapy with surgery, most of the patients relapse. Indeed, therapies require not only crossing the blood brain barrier (BBB) but also reaching the tumor cells within the heterogeneous and constantly evolving brain microenvironment that hinders their efficacy. Hence, understanding the tumor-induced changes on the BBB and its response to therapies is critical to engineer the most effective targeted therapies.
Herein, we explored the changes in tumor-associated endothelial cells (ECs), the main component of the BBB, by single-nucleus RNA sequencing of GL261 tumour-bearing mice at early and late stages; and upon treatment with temozolomide, the standard of care for these tumors, and with a promising cell-penetrating peptide that inhibits the oncoprotein Src, TAT-Cx43266-283.
By comparing the EC landscape from tumors at the two timepoints, we observed that ECs adopt a more activated phenotype, by the increase in the adaptive immune response and antigen presentation; show a significant boost in translation by the increase in ribosome assembly; and metabolic pathways, such as fatty acid metabolism and regulation of lipid transport. Temozolomide treatment, however, decreased the inflammatory response, and augmented the regulation of calcium and ion transmembrane transport, both crucial for barrier function.
Interestingly, early tumor-associated ECs from mice treated with TAT- Cx43266-283, compared with ECs from those treated with temozolomide, showed a decrease in pro-migratory pathways; and essential pro-angiogenic pathways such as WNT, hinting at a delay in the onset of the proangiogenic vascular phenotype.
Therefore, this study shows that glioblastoma triggers a more intricate blood vessel machinery than just an angiogenic response, and that a profound insight into the vascular remodelling induced by both glioblastoma and therapies is needed to ultimately impact the prospects of the disease.

Deciphering partial reprogramming mechanisms in mature neurons: implications in healthy ageing

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The discovery of the Yamanaka Factors (YFs)—OCT4, SOX2, KLF4, and MYC—has revolutionized cellular biology by demonstrating their capacity to induce pluripotency, reverse molecular signs of aging, and reprogram somatic cells. While much of the research has focused on peripheral tissue regeneration, the role of YFs in the central nervous system, particularly in the context of aging and healthy aging, remains largely unexplored. Here we use profiling experiments, neuronal assays, and behavioral tests to examine the impact of YFs induction on mature neurons in the cerebral cortex of both young and aged mouse brains. These results could reveal age-related changes driven by partial reprogramming, providing new insights into the mechanisms by which YFs mitigate the effects of aging and highlight their potential for neuronal rejuvenation.

Determination of the apolipoprotein E (APOE) gene polymorphism as a determinant of genetic risk for Alzheimer's disease: Analysis in a population of older adults in Castile and Leon (Spain)

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Alzheimer's dementia is a progressive neurodegenerative disease that affects memory due to genetic and environmental factors. A well-known gene that influences the risk of Alzheimer's disease (AD) is the apolipoprotein E (APOE) gene, which encodes for apolipoprotein E (ApoE), a protein mediating the binding of lipoproteins and other lipid complexes to specific cell-surface receptors. APOE gene has several alleles naming ε2, ε3 and ε4, encoding for the corresponding isoforms of ApoE, the ApoE4 having been associated to the development of more amyloid plaques leading to AD. However, the prevalence and risk associated with APOE vary across populations, therefore, more research is needed to better understand how population genetics may influence the risk of AD.
We aimed to study the potentiality of APOE polymorphism as biomarker of higher risk for suffering AD through a population screening in patients with clinical diagnosis of AD in the region Castile and Leon (Spain), by performing an observational case-control study. Saliva samples were collected and the APOE gene was analyzed by PCR and agarose gel electrophoresis, respecting ethical criteria.
The most prevalent allele in the AD population of Castile and Leon was ε3 (74.12%), followed by ε4 (22.57%) and ε2 (3.31%), APOE4 being much more common in AD than in control group. It was also observed that the ε2/ε2 genotype was not found in any individual with AD, but was found in healthy subjects, the opposite being observed for the ε4/ε4 genotype. The odds ratio indicated a four-fold increased risk of AD if having ε4 allele. The demonstrated relation between the different isoforms of APOE gene and the likelihood of developing AD has led to its consideration as a biomarker and a potential factor for pre-symptomatic therapy. The molecular mechanisms that confer a disruptive and protective role to ApoE4 and ApoE2, respectively, require further investigation.

Determining cellular composition of the spinal cord from single-cell transcriptomics data: individual variability, biases and contributing factors

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Cellular death is a central event in spinal cord injury (SCI) pathophysiology and a major determinant of the resulting functional deficits. Single cell RNA sequencing (scRNAseq) data comprises information on the cell composition that can be employed to infer population dynamics under different conditions. Several studies have applied a variant based on the sequencing of individual nuclei (snRNAseq) to build cellular atlases of the spinal cord establishing the abundance of each cell population. Here we develop a metaanalysis to estimate the individual variability in cell composition and the factors that condition it. Raw sequencing data from 5 studies of mice spinal cord (16 individuals) available at public repositories were aligned and mapped to the last reference genome comprising >50,000 coding and non-coding genes. The resulting count matrices were subject to quality controls and processed with standard pipelines for sample integration and cell classification and annotation. Results reveal that all samples share comparable cell type compositions. Proportions broadly agree with previous results but overestimate neurons with respect to histological and isotropic fractionator analyses. Individual variability in the abundance of each cell type is evident. Variations are associated to study-dependent factors, such as the employed chemistry, but also to study-independent ones such as the abundance of particular cell types or the total number of cells in the sample. Sample integration using Harmony to uniform sample information and reduce batch effects, reduces variability but modifies the identified cell types. Incorporation of information on expression of non-coding genes did neither affect the identified cell types nor reduced interindividual variability. In conclusion, snRNAseq data provide consistent information on spinal cell composition that can be employed to analyse cell death following SCI, but requires fine-tuning the data analysis techniques and considering relevant non-biological sources of variation.

Development and Validation of an In-Vivo Preclinical Model of Schizophrenia and Periodontitis

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Schizophrenia is a severe mental illness characterized by cognitive, affective and psychotic symptoms. Growing evidence implicates neuroinflammation in its pathophysiology. The neurodevelopmental theory of schizophrenia proposes a “double-hit model”: the first hit occurs pre/perinatally, causing neurodevelopmental alterations, while the second hit during late adolescence triggers the first psychotic episode. Periodontitis is a chronic inflammatory gum disease driven by oral dysbiosis whose progression is irreversible. Emerging studies suggest comorbidity between both pathologies, with chronic low-grade inflammation as a shared pathological mechanism.

This study aimed to develop and validate an animal double-hit model of schizophrenia and periodontitis and to explore neuroinflammatory alterations in the nervous system of the animals. To create this model, Poly I:C was administered to pregnant Wistar Hanover rats on gestational day 14, and periodontitis was subsequently induced in the offspring through dental ligatures and oral rinses with a solution containing periodontal bacteria (Porphyromonas gingivalis and Fusobacterium nucleatum) for 4 weeks. Animals were assigned to four groups (17-21 animals/group): schizophrenia and periodontitis, periodontitis alone, schizophrenia alone and control. Behavioral validation was performed using the Y-maze and Elevated Plus Maze (EPM) tests.

In the Y-maze test, both schizophrenia groups exhibited a lower discrimination index and reduced spontaneous alternation, indicating working memory impairments. In the EPM test, schizophrenia alone animals displayed reduced anxiety-like behavior, evidenced by increased entries into the open arms, whereas periodontitis alone animals avoided the open arms. Behavioral tests revealed significant sex differences, with females displaying more erratic exploration and hyperactivity. Periodontitis was validated through clinical variables, which confirmed increased oral inflammation in affected groups. To further validate the model, molecular biology analyses (Western blot, RTq-PCR and ELISA) are currently being conducted.

This novel preclinical model may provide valuable insights into the neuroinflammatory alterations underlying the comorbidity between schizophrenia and periodontitis, paving the way for future therapeutic strategies.

Differential Effects of Scn1a Dysfunction in the Prefrontal Cortex During Adolescence vs Adulthood

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The voltage-gated sodium channel Nav1.1, encoded by the SCN1A gene, plays a key role in generating and propagating action potentials, particularly within GABAergic system. Alterations in Nav1.1 function are linked to severe neurological disorders like Dravet syndrome or Alzheimer disease. Recently, we have shown that Nav1.1 dysfunction in the medial prefrontal cortex (mPFC) during adolescence leads to epilepsy, cognitive deficits, and depressive-like behaviors in adulthood.

To investigate the pathophysiological mechanisms linked to Nav1.1 dysfunction during mPFC maturation, we inactivated this channel at different developmental stages. Behavioral tests, such as Y-maze and fear conditioning extinction, revealed that mPFC-dependent cognitive functions remained intact in adulthood-inactivated mice. In contrast, both adult- and adolescent-inactivated groups exhibit epileptic and depressive-like phenotypes. Furthermore, abnormalities in mPFC and dHPC oscillatory activity were observed. Adolescent inactivation resulted in reduced delta and increased theta activity in the mPFC during the active state, whereas adult inactivation led to elevated delta in mPFC and theta in dHPC during resting and active states. Both groups showed reduced prefrontal-hippocampal coherence at delta frequency.
Differences in basal synaptic transmission and LTP plasticity of the mPFC were also analyzed. In adult-inactivated mice, the Nav1.1 agonist Hm1a did not alter LTP at L2/3-L2/3 synapses of the prelimbic PFC, as treated and untreated slices exhibited comparable responses to theta burst stimulation. In contrast, adolescent-inactivated mice showed a distinct response, failing to exhibit LTP in the presence of Hm1a. These findings highlight a developmental difference in Nav1.1 function at L2/3-L2/3 synapses of the prelimbic PFC.

Overall, the results suggest that Nav1.1 function is required for the correct maturation and/or activity of the mPFC circuitry implicated in cognitive tasks. In contrast, during adulthood, Nav1.1 activity becomes dispensable for cognition but remains important for emotional control and a balanced brain activity.

Direct differentiation of fibroblasts into neurons as a new neuronal model for the study of Friedreich's Ataxia

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Friedreich's ataxia (FA) is a rare autosomal recessive neurodegenerative disease characterized by progressive cerebellum degeneration and early atrophy of the spinal cord. It is caused by a GAA triplet repeat expansion within the first intron of the FXN gene, leading to a reduced expression of the frataxin protein (FXN). This protein is mainly located in mitochondria, where it plays a crucial role in the biogenesis of FeS clusters, as well as in the iron metabolism. The disease has no cure and one of the causes is due to the lack of robust experimental models that accurately replicate the human disorder. In order to better understand the disease and to have a platform to facilitate drug screening, novel animal and cell model are actively being developing, using different technical approaches.

In this study, we have generated a new FA neuronal model based on the direct transdifferentiation of human fibroblasts from FA-patients and control subjects to induced neurons (iNeurons). These iNeurons display a neuronal-like phenotype, expressing some typical markers of mature neurons such as MAP2 and phosphorylated NF proteins, while fibroblast-specific genes suffer a strong down-regulation. Furthermore, compared to those iNeurons differentiated from healthy fibroblasts, FA-patients' iNeurons show lower FXN levels and reduced expression of proteins containing FeS clusters such as Aconitase 2 and some complexes of the mitochondrial electron transport chain. This may potentially affect mitochondrial function, as corroborated by some proteins involving mitochondrial turnover and homeostasis, whose levels change in this model.

Therefore, our approach shows that the direct differentiation of mature fibroblasts from FA patients into neurons is a viable strategy. The resulting FA iNeurons recapitulate key hallmarks of the disease, providing a valuable model to further use a drug screening platform.

DIRECT LINEAGE REPROGRAMMING OF HUMAN ASTROCYTES INTO INDUCED NEURONS

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The advent of direct lineage reprogramming has opened an unprecedented perspective for lost neurons' replacement following injury and neurodegenerative diseases. Successful murine astroglia reprogramming has shown the feasibility of converting murine astroglia both in vitro and in vivo. However, it remains unknown whether such lineage reprogramming can also be achieved in the case of human astrocytes which differ markedly from their rodent counterparts.

To tackle this question, we have differentiated human astrocytes from human induced pluripotent stem cells (hiPSCs) using a genome-edited hiPSCs line. Astrocyte differentiated during two weeks have been challenged in vitro to the overexpression of the proneural transcription factor Neurog2. After Neurog2 expression, cells displaying neuronal morphology and expressing neuronal markers βIIItubulin were detected. In order to assess the physiology of the human astrocyte-derived induced neurons, we carried out patch clamp recordings. Neurog2-derived induced neurons were able to fire action potentials confirming their neuronal identity. Finally, time-lapse imaging experiments confirmed the lineage conversion of human astrocytes into induced neurons in vitro.
To further investigate human astrocyte reprogramming in a human tissue-like context, we have generated human cerebral organoids. Taking advantage of the genome-edited hiPSCs line, we have targeted human astrocytes in cerebral organoids to overexpress Neurog2. After overexpression of Neurog2, we found cells expressing the neuronal marker βIIItubulin that could be traced back to cells of astroglia origin. Altogether, our results show that Neurog2 is able to directly reprogram human astrocytes into induced neurons, in a 2D and 3D context.

Disrupted Cholesterol Pathways in TDP-43 Proteinopathies: A Crucial Contributor to Myelin Dysfunction

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TDP-43 is a nuclear protein encoded by the TARDBP gene, which forms cytoplasmic inclusions in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). These diseases involve multiple pathological mechanisms, with alterations in lipid regulatory mechanisms as an emerging key factor. However, little is known about the role of TDP-43 in lipid metabolism regulation and its potential link to myelin dysregulation, given that nearly 70% of myelin is composed of lipids, with cholesterol being the most critical component.
To investigate the role causal role of TDP43 dysfunction in the alteration of brain cholesterol homeostasis, we analyzed frontal cortex tissue from a TardbpM323K/M323K knock-in mouse model using immunohistochemistry and multiomics (lipidomics, and transcriptomics), including functional splicing analyses. To validate our findings beyond this specific mutation, we examined post-mortem frontal cortex tissue from FTLD-TDP patients through immunohistochemical staining, and performed analyses of transcriptomic and lipidomics patients' databases.
Our results show that TDP-43 pathology leads to transcriptional dysregulation of genes essential for myelin production and maintenance. Our lipidomic analysis revealed alterations in lipid pathways involved in membrane composition and lipid droplets accumulation, particularly affecting cholesterol-related species. Further studies showed impaired cholesterol accumulation via lipid droplet formation, decreased endogenous cholesterol synthesis, and increased cholesterol transport. These findings suggest that TDP-43 alteration disrupts cholesterol homeostasis, potentially affecting myelin integrity. This opens new avenues for therapeutic and diagnostic strategies targeting lipid metabolism in TDP-43 proteinopathies.

DISTRIBUTION OF PATHOLOGICAL PROTEINS IN THE HUMAN ENTORHINAL CORTEX, OLFATORY PEDUNCLE AND SUBVENTRICULAR ZONE IN ALZHEIMER´S DISEASE

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Alzheimer's Disease (AD) is an idiopathic neurodegenerative disorder that progressively affects regions of the brain in a sequential and predictable manner, with the entorhinal cortex and the olfactory pathway being one of the first areas to be affected. Its pathophysiology is characterized by the accumulation of neurofibrillary tangles of hyperphosphorylated tau protein and extracellular β-amyloid plaques. The olfactory system plays a fundamental role in the detection, integration, and processing of volatile and non-volatile chemical stimuli from the environment. In recent years, interest in this system has increased due to the olfactory dysfunction observed in several neurodegenerative diseases. These dysfunctions often precede the appearance of more characteristic clinical symptoms by decades. Our study focused on analysing the distribution of glial and neuronal populations and proteins associated with AD in the entorhinal cortex, and along the olfactory peduncle (poorly studied to date), up to the subventricular zone, one of the most important neurogenic niches in the adult mammalian brain, which is virtually connected to this region. For this purpose, four cases were included in the study: two diagnosed with mild and two with moderate AD. The tissue was processed using conventional histological techniques and immunofluorescence targeting relevant markers, including β-amyloid, tau, Neu-N, Iba1, GFAP, and nestin. The analysis was performed using confocal microscopy, allowing a detailed study of the distribution and structure of the elements expressing these markers. Stereoinvestigator will be used to estimate the number of each population and the amount of pathological protein aggregates. The results allowed us to know the distribution and cellular interaction in each region, as well as the presence of pathological proteins. This work aims to shed light on how the neurodegenerative process affects two of the first areas involved in the AD, and how this may affect the subventricular zone in terms of neuroplasticity.

Divergent Effects of APOE3 and APOE4 Human Astrocytes on Key Alzheimer's Disease Hallmarks in Chimeric Mice

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Despite strong evidence supporting that both astrocytes and apolipoprotein E (APOE) play crucial roles in the pathogenesis and progression of Alzheimer's disease (AD), the impact of astrocytes carrying different APOE variants on key AD pathological hallmarks remains largely unknown. To explore such effects in a human-relevant context, we generated a chimeric model of AD. We transplanted isogenic APOE3 or APOE4 human induced pluripotent stem cell (hiPSC)-derived astrocyte progenitors into neonatal brains of AD model mice. We show that five to six months after transplantation, transplanted cells have differentiated into mature astrocytes (h-astrocytes) that often integrate in upper layers of one cortical hemisphere. Remarkably, APOE3 and APOE4 h-astrocytes differentially affect main AD pathological hallmarks. While APOE3 h-astrocytes ameliorate Aβ pathology, Tau pathology and neuritic dystrophy, APOE4 h-astrocytes aggravate these AD processes. Moreover, they modulate microglia responses to Aβ pathology in opposite ways. APOE4 h-astrocytes and hAPOE4 enhance microglia clustering around Ab plaques and exacerbate DAM state. In contrast, APOE3 h-astrocytes and hAPOE3 reduce clustering and induce a more homeostatic state on plaque associated microglia. These findings highlight a critical contribution of h-astrocytes to key AD hallmarks in chimeric mice. Moreover, our results reveal that h-astrocytes with different APOE variants and the different forms of APOE they secrete have a crucial role in AD progression. Now, to determine the molecular differences between APOE3 and APOE4 h-astrocytes, we are doing bulk RNA sequencing of the h-astrocytes in AD and wild type chimeric mice.

DMRTA1 SNPs association in the genetic susceptibility to ADHD

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Attention deficit hyperactivity disorder (ADHD) exhibits a strong genetic component and a marked sex bias, suggesting that sexual differences in neuronal network development might influence the etiology and sex-specific vulnerability or resilience to the disorder. However, the contribution of genetic mechanisms of brain sexual differentiation to neuropsychiatric disorders remains poorly understood.
Doublesex and Mab-3 Related Transcription Factors (DMRTs) stand out for their involvement in the control of sexually dimorphic traits among phylogenetically distant species. Although they are evolutionarily conserved, their function in mammalian brain sexual differentiation remains largely unexplored. In humans, DMRTA1/Dmrt4 and DMRTA2/Dmrt5 mutations have been linked to severe neurodevelopmental disorders such as microcephaly and lissencephaly. Recent studies from our laboratory based on a genome-wide association study (GWAS) using Psychiatric Genomics Consortium (PGC) data identified significant associations between regulatory variants in DMRTA1 and DMRTA2 and ADHD. DMRTA1 ADHD-associated variants are located in the promoter region, forming a linkage disequilibrium (LD) block. Four haplotypes have been identified in non-Finnish European and Spanish populations, with Haplotype 4 being the rarest and associated with increased ADHD risk. This study investigates the functional effects of DMRTA1 promoter haplotypes on gene expression in neuroblastoma cell lines and explores transcription factor binding sites, aiming to elucidate the potential link between genetic regulation of DMRTA1 and ADHD susceptibility, particularly in the context of sexual dimorphism in brain development.

Early synaptic changes and reduced brain connectivity in a PD-like mouse model with depressive phenotype

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Parkinson's disease (PD) is a neurodegenerative disorder that causes both motor and non-motor symptoms. Between the latter, anxiety and depression are highly prevalent, significantly impacting quality of life and disease progression. A key pathophysiological hallmark of PD is the aggregation and accumulation of misfolded α-Synuclein (α-Syn) throughout the body-brain axis. Early in the disease, depressive symptoms have been linked to accumulated α-Syn in serotonin (5-HT) neurons in the raphe nuclei, but their relationship to broader brain changes remains unclear.

In this study, we combined functional magnetic resonance imaging (rs-fMRI) along with synaptic and cellular activity mapping in a mouse model with human α-Syn overexpression in the dorsal raphe nuclei, which exhibits a depressive phenotype (D-PD model). We explored how α-Syn accumulation in the brain's 5-HT system disrupts synaptic function and connectivity in regions involved in emotional regulation. Additionally, we confirmed these synaptic changes in human brain samples.

Our results showed that α-Syn accumulates in raphe 5-HT, dopaminergic, and GABAergic neurons, spreading to efferent areas and causing axonal pathology. In parallel, a progressive synaptic dysfunction was evidenced by alterations in key synaptic markers: decreased post-synaptic MAP-2 and PSD95, alongside increased presynaptic SV2A, synaptophysin, and VAMP2, as well as the motor protein dynein in interconnected brain areas (e.g. CPu, and hippocampus). Similar alterations were observed in human brain tissue. We also detected increased Egr-1-dependent cellular activity in cortical and subcortical areas at 8 weeks post-AAV infusion, while activity in the DR decreased by 4 weeks. rs-fMRI studies further revealed region-specific disruptions in resting-state brain activity, with reduced functional connectivity in cortical-limbic-raphe networks, as reported in patients with depression and PD.

Taken together, our findings provide evidence for potential biomarkers associated with α-Syn pathology in emotional brain circuits, facilitating a better understanding of the possible mechanism underlying depression in PD.

EFFECT OF APOE POLYMORPHISM AND G206D-PSEN1 MUTATION ON HIPPOCAMPAL NEURONS AND BRAIN ORGANOIDS FROM ALZHEIMER´S DISEASE PATIENTS

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Alzheimer's disease (AD) is characterized by progressive neurodegeneration of the primary brain regions involved in memory function, the entorhinal cortex and hippocampus. The human APOE polymorphism, more specifically the presence of the ε4 allele (coding the APOE4 protein isoform), represents a genetic form of late-onset AD, while mutations in the PSEN1 gene are responsible for many cases of early-onset familial AD. There is mounting evidence implicating APOE4 in multiple facets of AD pathogenesis; however, the full scope of its impact on human neuronal maturation, function, and degeneration remains to be fully elucidated. Furthermore, the effect of the G206D-PSEN1 mutation on human neurons remains to be explored in depth. To address this, we have obtained human hippocampal neurons and brain organoids by adding small molecules and growth factors to isogenic and non-isogenic lines of induced pluripotent stem cells (iPSCs). These iPSCs were derived from fibroblasts of AD patients carrying the ε3 or ε4 alleles (in homozygosis), patients carrying the G206D-PSEN1 mutation and healthy individuals. The iPSC-derived neurons expressed hippocampal markers and demonstrated a functional profile, as evidenced by glutamate release, electrical activity, and synapse formation, as determined by electron microscopy and synaptic bouton analysis. Furthermore, the role of APOE4 in neurodegeneration was confirmed by detecting amyloid-beta 42/40, total Tau, and phosphorylated Tau in the culture medium, as well as by the presence of an increased number of extracellular amyloid-beta-like plaques and intracellular p-Tau181 aggregates. Finally, APOE polymorphism also affected neuronal morphology and the number of synaptic boutons. Collectively, these findings underscore the distinct impact of APOE polymorphism and G206D-PSEN1 mutation on neuronal maturation, dysfunction, and neurodegeneration in AD. Subsequent analyses on brain organoids will facilitate the identification of specific AD phenotypes within a more physiological framework, thereby enhancing our understanding of the complex interplay between APOE and PSEN1 genetics and neurodegeneration.

Effect of GABAergic precursor transplantation in a murine model of Syntaxin Binding Protein 1 (STXBP1) syndrome

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STXBP1 syndrome, an early infantile epileptic encephalopathy, is characterized by severe seizures, intellectual disability, developmental delay, and autistic-like behaviour. It is mostly caused by mutations in the gene STXBP1, encoding for Munc18-1 protein, a member of the SNARE complex essential for synaptic vesicle release. Munc18-1 dysfunction mainly affects GABAergic interneurons, altering the excitatory/inhibitory balance and leading to neuronal hyperexcitability, which manifests as recurrent seizures. Treatment options are exclusively symptomatic, and therapeutic alternatives are urgently needed.

In order to investigate a possible cell-based therapeutic strategy targeting the GABAergic system, we transplanted GABAergic neuronal precursors derived from the medial ganglionic eminence (MGE) in Stxbp1+/- haploinsufficient mice at different ages. Analysis at behavioural, electroencephalographic, and histological level showed that perinatal transplantation increases survival rates up to 60%, reducing seizures frequency and severity. Additionally, perinatal and adult transplantation leads to complete recovery (perinatal) or partial improvement (adult) of cognitive function and behavioural deficits. Anxiety levels were significantly reduced in open field, elevated plus maze and dark-light tests. Contextual associative memory was also significantly improved, as observed in the fear conditioning test, while cued memory was not recovered. Finally, nesting test points that autism-related behaviour was also significantly reduced.

Taken together, our results suggest that GABAergic precursor cell transplantation represents a promising therapeutic strategy with potential clinical applications for the treatment of STXBP1 syndrome at early and late stages.

Effect of Metalloproteinase Inhibitor ACT-03 in Traumatic Brain Injury-derived Epilepsy

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Traumatic Brain Injury (TBI) results from a head impact that triggers acute cell death and inflammation, often leading to long-term neurodegeneration, epilepsy and cognitive deficits. Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, are involved in post-TBI inflammation and tissue remodeling which might contribute to epileptogenesis. In this study, we evaluated the safety and effects of ACT-03, a selective MMP-2 inhibitor, by analyzing edema by MRI, and the expression of MMP-related genes and proteins. We hypothesized that TBI would show increased neuroinflammation and MMP-2/MMP-9 expression compared to SHAM controls, and that ACT-03 treatment would mitigate these effects while reducing the likelihood of epilepsy development. In the short term (1-week of treatment after TBI), qPCR analysis showed no significant differences in pro-neuroinflammatory markers (Il6, Il1b) and cell death indicators (TNFα) between treated and untreated groups, while MMP expression remained unchanged at the time point of analysis, western blot confirmed MMP inactivation. MRI analysis demonstrated that ACT-03 did not exacerbate the TBI-triggered edema. We are currently evaluating the long-term effects of ACT-03 treatment on the induction of seizures by TBI in the long term using intracranial electrodes.

Effects of caloric restriction and sex-specific influences on spatial memory in 6-month-old TgF344-AD rats

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Alzheimer's disease (AD) is a leading cause of cognitive decline in older adults, with aging as the primary risk factor. Nevertheless, accumulating evidence underscores the significant role of lifestyle factors, i.e. diet, in modulating the onset and progression of AD-related pathology. This study investigates how dietary interventions influence aging and AD-related mechanisms, emphasizing on sex-specific differences. Among these interventions, caloric restriction (CR) — a reduction in caloric intake without malnutrition — has shown promise in counteracting cognitive deterioration by modulating epigenetic regulation, neuroinflammation, synaptic plasticity, and metabolic processes. Here, we examined whether CR can mitigate early cognitive deficits in male and female wild-type (WT) and TgF344-AD rats, a transgenic model of AD carrying human APP and PSEN1 mutations. Animals were randomly assigned at 2.5 months of age to either CR or ad libitum feeding. Spatial memory, a hippocampus-dependent function highly sensitive to aging and AD pathology, was assessed at 6 months using the Morris Water Maze (MWM), following an acquisition phase of four daily trials over five consecutive days and a probe test conducted 72 hours later. Preliminary results suggest an age-related decline in spatial memory, particularly in TgF344-AD rats of both sexes. Although all animals successfully learned the task, transgenic males showed lower performance during acquisition, while cognitive flexibility appeared more affected in transgenic females, indicating sex-specific vulnerabilities. Interestingly, CR seems to attenuate these effects in both sexes, with a more pronounced benefit in males — possibly because aging has a greater impact on males, making them more responsive to the intervention. These initial findings support CR as a promising non-pharmacological strategy to preserve cognitive function, although sex differences in aging trajectories may influence its effectiveness. Future studies will explore the mechanisms underlying these effects, including potential modulation of hippocampal plasticity, neuroinflammation, and metabolic signaling pathways involved in AD.

Effects of Dietary Modification on Brain Oxidative Stress Status and Cognitive Function in Rats Previously Exposed to a High-Fat Diet

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A high-fat diet (HFD) is a key contributor to metabolic syndrome that has been linked to neurocognitive decline, primarily due to increased neuroinflammation and oxidative stress in the brain. This study aimed to evaluate the effects of dietary modifications on oxidative stress markers in the parieto-occipital cortex of rats exposed to a HFD and dietary interventions.
Five-month-old male and female Sprague-Dawley rats were fed an HFD for three months, followed by a two-month dietary intervention in a subset of animals, switching to either a standard or antioxidant-enriched diet. While all HFD-fed rats developed obesity hyperglycemia and hepatic lipid deposits, cognitive impairment was more pronounced in females, who required more time to complete the Barnes maze test (learning and memory). In contrast, males exhibited significant deficits only in cognitive flexibility during the re-learning phase. Notably, dietary interventions did not significantly improve Barnes test performance.
Oxidative stress analysis in the parieto-occipital cortex revealed increased lipid peroxidation (malondialdehyde levels) and decreased enzymatic activity of superoxide dismutase and catalase in HFD-fed animals of both sexes. These alterations were significantly reversed by dietary interventions, suggesting enhanced antioxidant capacity. However, glutathione peroxidase (GPX) activity was unexpectedly higher in HFD-fed males and in both sexes following dietary interventions, whereas glutathione reductase (GRd) activity increased only in males after dietary changes.
These findings indicate that while an HFD increases oxidative stress in the brain cortex of both sexes, females are more susceptible to its detrimental effects on cognitive function. Moreover, transitioning to a healthier diet for two months effectively mitigates oxidative stress in the cortex, though this improvement does not translate into cognitive recovery.
M. J.-G. was supported by a FPI grant (FPI_062_2021, Govern de les Illes Balears, Spain).

Effects of voluntary physical exercise on plasma myokine levels after traumatic brain injury in late middle-aged male and female rats

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Physical exercise can reduce memory deficits associated to traumatic brain injury (TBI) in young rats through varied neuroprotective and neuroplastic mechanisms. Some effects of exercise on the brain are mediated by muscle-to-brain signalling, via muscle-secreted growth factors and cytokines known as myokines. However, little is known about these effects in older rats. We therefore examined the effects of TBI and exercise on plasma myokines in late middle-aged (14 months old) rats of both sexes. The animals went through a TBI (controlled cortical impact) or sham surgery, followed by 7 weeks of either a sedentary condition or voluntary wheel running exercise. Levels of brain-derived neurotrophic factor (BDNF), fibroblast growth factor-21 (FGF-21), secreted protein acidic and rich in cysteine (SPARC), irisin, vascular-endothelial growth factor (VEGF) and interleukin 6 (IL-6) were measured in plasma before TBI, 1 week after injury (before exercise), and 7 weeks after injury. Female rats ran more than males regardless of injury status. While male TBI rats ran consistently less time and distance than sham-operated animals, female TBI-operated rats exhibited the opposite behaviour. Exercise reduced FGF21 levels in both sexes, and increased BDNF in sham and TBI males (but only in TBI females), compared to the sedentary groups. VEGF levels increased in response to exercise in TBI females only. Exercise had no significant effect on the levels of either IL-6 or irisin, but irisin was reduced after TBI. Sex differences were observed in IL-6 and irisin myokines, since IL-6 was reduced in males and irisin in females. Finally, no effects of exercise, TBI and sex were found on SPARC levels. In conclusion, in late middle-aged TBI-injured rats exercise modified the levels of BDNF, FGF21 and VEGF, but not those of IL-6, irisin and SPARC. Myokine response to exercise after brain injury showed also sex and TBI differences.

Efficacy of the intranasal route for the administration of NPY1R and its effects on the treatment of neurodegenerative and mood disorders via heteroreceptor complexes formation

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Dysregulation of adult hippocampal neurogenesis has been implicated in currently highly prevalent neuropsychiatric disorders such as major depressive disorder (MDD) or cognitive decline such as Alzheimer's disease (AD). The neuropeptide Y1 receptor (Y1R) of neuropeptide Y (NPY) has been found to interact with galanin receptor 2 (GALR2) in certain areas of the hippocampus with effects on memory processes and mood-related behaviour within 24 hours of intranasal administration. This study addresses the synergistic effects of the long-term NPY1R-GALR2 interaction on both mood-related behaviour and cognitive function following intranasal co-administration, as well as exploring other interactions of NPY1R. These changes have been assessed at molecular, cellular and behavioural levels.

GALR2 and/or NPY1R agonists were administered intranasally, while other cohort of rats received ketamine and/or NPY1R agonist. Behaviour was assessed using the Forced Swimming Test (FST) to analyse antidepressant activity and the object-in-place task for spatial memory. In brain slices, we performed in situ PLA techniques to detect GALR2-NPYY1R and NPY1R- TrkB heteroreceptor complexes formation, as well as different immunolabelling techniques with proliferating cell nuclear antigen (PCNA), doublecortin (DCX), bromodeoxyuridine (BrdU) and brain-derived neurotrophic factor (BDNF).

The results show that the interaction of NPYY1R with both GALR2 and TrkB enhances antidepressant behaviour and spatial memory, while increasing the formation of heteroreceptor complexes (GALR2-NPYY1R or NPY1R-TrkB) in the dentate gyrus of the hippocampus. Both treatments promote neuroblast proliferation, survival as well as neuronal differentiation and maturation. In addition, co-stimulation of NPY1R-TrkB enhances BDNF expression.

Our results contribute to the understanding of neuroplasticity mediated by the formation of NPY1R-GALR2 and NPY1R-TrkB heteroreceptor complexes following intranasal administration of NPY1R and GALR2 agonists, highlighting their role in enhancing the processes of neurogenesis, neuronal survival and maturation. These findings open up new therapeutic approaches for the treatment of MDD and AD.

Emerging role of endosomal NHE7 in synaptic function and neurodevelopmental disorders

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Neurons exhibit intricate morphology and compartmentalization, necessitating precise organelle positioning and protein trafficking. Additionally, synapses represent exceptional subcellular compartments, adding facets to the complexity of membrane trafficking systems to meet synaptic demands. Accordingly, disturbances in biosynthetic and endolysosomal processes contribute to neurodevelopmental and neurodegenerative disorders characterized by synaptic deficits, ultimately leading to cognitive impairments. The factors underlying neuronal dysfunction in those endolysosome-related disorders are undoubtedly multifaceted. However, despite considerable progress in identifying these factors, a comprehensive understanding of their mechanisms remains elusive. Increasing evidence underscores the pivotal role of pH/ion homeostasis within endomembrane compartments in maintaining central nervous system function and its association with neurological disorders. The intracellular sodium-hydrogen (Na+/H+) exchangers (NHE) emerge as critical and largely under-explored transporters that collaborate to finely tune compartmental pH and ion content which is of essential importance for the physiology of the secretory and endocytic pathways and its diverse trafficking events. Few studies have addressed the function of NHE7 which has been associated with cancer and as a risk gene for Alzheimer's Disease. Additionally, mutations in SLC9A7/NHE7 cause Rare and X-linked non-syndromic intellectual disability (ORPHA: 101685 and 777). However, the specific role of NHE7 in neuronal function remains elusive. Here, we show that NHE7 acts as a proton-loading transporter in primary brain cells. Furthermore, we show for first time that NHE7 positive vesicles can localize at synaptic buttons, suggesting its potential involvement in regulating pH dynamics within these neuronal structures, being crucial for maintaining synaptic homeostasis. Taken together, our results shed new lights on the mechanisms underlying NHE7 function in vesicular ion homeostasis and synapse regulation, which eventually will help to better understand the pathobiology of intellectual disability, autism and other synapse-related disorders associated to NHE7 dysfunction.

Exacerbation of Tau-Associated Pathology following Peripheral Injection of Blood from a Tauopathy Model

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. It remains unknown how tau aggregates spread beyond the central nervous system through peripheral pathways, although there is evidence that Aβ can disseminate through prion-like mechanisms in both central and peripheral routes. Tau aggregates have been detected in biological fluids and peripheral tissues in mice and humans, and current evidence suggests that tau can also propagate in a prion-like manner. Nevertheless, the role of peripheral tau aggregates in the development of tau-related disorders, including AD, is poorly understood. In this study, we explore whether peripheral administration of blood from aged P301S tau transgenic mice, a well-established tauopathy model, worsens tau-associated pathology in the brain. Young P301S mice received blood via both intraperitoneal and intravenous injections, and were evaluated through behavioral, biochemical, and histological analyses to assess brain tau-associated pathology. Our results reveal that mice receiving blood from aged tau transgenic donors exhibited significantly enhanced tau deposition in the hippocampus, along with an increased glial inflammatory response. In addition, recipient mice displayed motor impairment, indicating a worsening of neurodegenerative processes. Overall, these findings provide compelling evidence that blood from tau mice may contribute to the progression of tau-related neurodegeneration. Unraveling the mechanisms behind peripheral tau dissemination could offer novel insights into disease progression and identify potential biomarkers and therapeutic targets for AD and other tauopathies.

Exploring a neuroprotective role for the transcription factor REST in Parkinson's Disease in vivo

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The expression of the Repressor Element 1-Silencing Transcription (REST) factor is induced in the aging human brain, and it has been shown to repress cell death and promote cellular stress response in healthy aging. On the other hand, REST deletion was shown to lead to age-related neuronal loss in a conditional mouse knock-out model. In neurodegenerative pathologies, such as Dementia with Lewy Bodies (DLB), REST is lost from the nucleus; moreover, we have reported dysregulation of REST and its target genes in Parkinson's Disease (PD) models. A recent study suggests that the recruitment of REST to Lewy Bodies may prevent its neuroprotective functions in PD. This evidence highlights REST as an interesting target for PD treatment, promoting the repression of cell death, the reduction of oxidative stress and supporting mitochondrial health, which are dysfunctional in PD. The aim of this study was to confirm or discard REST as a target for PD in human SNCA-overexpressing (SNCA-OVX) mice and in the intracranial PFF (alpha-synuclein Pre-Formed Fibrils) injection model. REST overexpression was achieved through viral injection, and its effects on dopaminergic cell counts, protein aggregation, mitochondrial function, as well as on motor and cognitive performance were investigated. Collectively, our data showed a restorative effect in alpha-synuclein pathology and mitochondrial health by REST on the neurons within the Substantia Nigra pars Compacta (SNc) and the Ventral Tegmental Area (VTA). Interestingly, this was accompanied by a reduction in SNc neurons and subtle motor defects. In the SNCA-OVX animals, this was also accompanied by the rescue of anxiety phenotypes and the neuronal population of the VTA. Together our interpretation of the data is that high levels of REST, such as the ones perhaps lost in the lost population of the SNc, are toxic, while low levels of REST can be neuroprotective.

Exploring Aberrant Hippocampal Neurogenic Response and Cognitive Impairment in a Thrombotic Stroke Mouse Model

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Stroke is the second leading cause of death and a primary contributor to vascular cognitive impairment and dementia worldwide. Previous research using a mouse model of brain ischemia with permanent occlusion of the middle cerebral artery (pMCAO) and ipsilateral carotid ligature demonstrated a neurogenic response in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). This response included aberrant post-stroke neuron morphology linked to cognitive deficits. In this study, we examined adult hippocampal neurogenic niche dynamics in a thrombotic stroke mouse model induced by FeCl3 application, sparing carotid blood flow and meningeal integrity. Two-month-old C57BL/6 mice underwent either pMCAO or sham surgery and hippocampal progenitor cells were analyzed via immunofluorescence at different time points. New integrated DG neurons after two months were assessed by BrdU injections. Another cohort was evaluated for acute (24h) and chronic (5-week) lesions using T2-magnetic resonance imaging and arterial spin labeling.

This model produced a cortical-restricted acute lesion, leading to long-term cortical atrophy and hypoperfusion. Despite the infarct did not affect the hippocampus, ipsilateral hippocampal hypertrophy occurred ($p<0.05$, n=5-9). An increase in SGZ neuroblasts was observed 7–30 days post-ischemia ($p<0.05$, n=4-6 per group), accompanied by aberrant morphologies in both hemispheres ($p<0.05$, n=5-6). Ischemic mice also exhibited cognitive deficits and hypoactivation of hippocampal granular neurons ($p<0.05$, n=8-17). FeCl3-induced stroke is a suitable and accessible experimental model for the study of adult hippocampal neurogenesis and that the defects in the latter are a common theme underlying post-stroke cognitive impairment.

Exploring Lysophosphatidic Acid as a Potential Therapeutic Strategy for Pelizaeus-Merzbacher Disease

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Pelizaeus-Merzbacher disease (PMD) is a rare and severe X-linked leukodystrophy characterized by white matter degeneration due to mutations or duplications in the Plp1 gene. These genetic alterations lead to the toxic accumulation of the proteolipid protein (PLP) in oligodendrocyte (OL) cell bodies, preventing its proper export to the myelin sheath. As a result, increased oxidative stress, mitochondrial dysfunction, and OL apoptosis occur, ultimately leading to hypomyelination, neuroinflammation, and a fatal clinical course. Despite ongoing research into potential therapeutic approaches, no effective treatment is currently available.
Lysophosphatidic acid (LPA) is a signaling molecule that exerts diverse biological effects through membrane receptors (LPA1–LPA6) and nuclear receptors (PPARγ). Notably, LPA plays a role in oligodendrocyte differentiation, mitochondrial function modulation, and oxidative stress reduction—mechanisms directly implicated in PMD pathogenesis. This suggests LPA as a promising candidate for therapeutic exploration. In this study, we investigate the potential of LPA pathway enhancement as a therapeutic strategy for PMD. We analyze the molecular mechanisms of LPA-mediated oligodendrocyte differentiation and evaluate its therapeutic effects in vitro using a Plp1-overexpressing mouse model (PlpTg66). Our findings provide insights into the potential benefits of LPA signaling modulation for mitigating PMD pathology. Moreover, this knowledge could shed new light on the treatment of other leukodystrophies and white matter disorders, offering hope not only for children affected by PMD but also for a broader spectrum of patients with neurodegenerative diseases. This work was financially supported by the Excellent Project from The Regional Ministry of Universities, Research and Innovation of Andalusia. PAIDI 2020 (ProyExcel_00996).

Exploring the antitumor effect of the cell-penetrating peptide TAT-Cx43mod in a murine model of tumor resection and its tumor microenvironment

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Glioblastoma (GBM) is the most aggressive and frequent form of primary brain tumor. Glioblastoma stem cells (GSCs), a subpopulation of cells within the tumor, are considered the main cause of GBM recur. Our laboratory has designed a cell-penetrating peptide, TAT-Cx43266-283, that reduces GSCs proliferation, migration and invasion and increases GBM-bearing mice survival by inhibiting the activity of c-Src oncoprotein. In this study we investigated the effect of a new peptide (TAT-Cx43mod) based on TAT-Cx43 in which some specifically directed modifications have been made to improve its antitumor efficacy. As GBM resection is the first-line treatment and several features of the GBM and its microenvironment are affected by this surgical procedure, we investigated the effect of TAT-Cx43mod in a murine model of GBM resection.
For these purposes, the murine GL261-GSCs were intracranially injected into the brains of immunocompetent C57BL/6 mice, and once the tumors had already developed, animals were subjected to GBM resection or sham operation and treated either with TAT-Cx43mod or saline until the end of the experiment. Immunofluorescence staining followed by confocal microscopy was performed in brain sections of these mice.
Although the results are preliminary, the effect of TAT-Cx43mod in combination with tumor resection improved mice survival. In fact, we analyzed the expression of TGFβ, an important GBM target, and we found that TAT-Cx43mod reduced its expression in specific locations related to blood vessels associated with microglia or macrophages. TGFβ contributes to the formation of tumor microtubes, which are known to participate in GBM invasion and are mainly composed of growth-associated protein 43 (GAP43). Indeed, the analysis of GAP43 showed a decrease inside the tumor upon treatment with TAT-Cx43mod.
Altogether, our results suggest that TAT-Cx43mod prolongs survival in a murine model of GBM resection and provide some clues about the effectors of its antitumor effect.

Exploring the Role of Metabolic Disruption in ALS Pathogenesis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by the progressive degeneration of motor neurons. The exact causes remain unknown and there are no effective treatments. However, genetic and environmental factors have been implicated in the onset and progression of the disease. This work aims to investigate the effects of metabolic stress, in particular glycolysis inhibition, in the context of the TDP-43-Q331K mutation, a pathogenic variant in humans, and its impact on homeostatic processes altered in ALS.

We will use both in vitro and in vivo approaches. In vitro, we use cortical primary neuronal cultures and immortalised fibroblasts (MEFs) derived from TDP-43-Q331K mouse models to evaluate the effects of glycolysis inhibition on cellular pathways relevant to ALS, including protein aggregation, RNA metabolism, mitochondrial function and stress granule dynamics. In vivo, we are administering 2-deoxy-D-glucose (2-DG) to TDP-43 Q331K mice to assess how metabolic imbalance affects disease progression at the systemic level. In addition, we analyse metabolic changes in MEFs carrying the FUS (Δ14) mutation, which is associated with juvenile ALS.

Finally, this work explores the role of metabolic stress in stress granule formation and its potential link to ALS pathogenesis. Through a multidisciplinary approach combining molecular biology, biochemistry and functional assays, we aim to gain deeper insights into the interplay between metabolic stress and TDP-43 dysfunction. This knowledge may help to identify novel therapeutic targets for ALS.

EXPLORING THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN SERPIN REGULATION: INSIGHTS INTO ALZHEIMER'S DISEASE AND POST-STROKE DEMENTIA

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Serpins constitute a family of proteins associated with various forms of dementia, including Alzheimer's disease (AD) and vascular dementia. Despite their relevance, their specific roles and regulatory mechanisms remain poorly understood. Our recent findings suggest that the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor influenced by gut microbiota (GM) and environmental contaminants, may drive a Serpin signature in reactive astrocytes. Here, we evaluate the role of AhR in the regulation of Serpins in AD and post-stroke dementia. For this purpose, constitutive AhR-KO mice were subjected to middle cerebral artery occlusion and/or crossed with APPNL-F mice to assess the role of AhR in the regulation of astrocytic serpins in post-stroke dementia and AD. Immunoinflammation, astrocityc reactivity and serpin expression were evaluated by immunofluorescence, WB, and qPCR. The role of AhR in regulating serpin expression was evaluated in astrocyte cultures upon AhR activation and/or inhibition using AhR agonists, such as FICZ, I3S and L-Kyn and the antagonist BAY-6096. Our data obtained through western blot and immunofluorescence, reveal an increase in serpin expression levels in astrocytes from both our murine model of AD and the vascular dementia model following stroke. This increase appears to be, at least in part, AhR-dependent, since AhR-KO mice in both pathological models exhibit a significant reduction in serpin compared to their respective controls. Promoter region analysis of different serpins suggests the presence of AhR response elements. Finally, AhR activation in astrocyte cultures using specific AhR agonists, leads to an increase in serpin levels, an effect that is blocked by AhR antagonist, suggesting that AhR regulates serpin mRNA levels. Taken together, our results suggest that AhR is a key regulator of serpins in astrocytes and may be involved in the inflammatory response in AD and post-stroke dementia. Understanding this relationship could provide new therapeutic strategies for these pathologies.

Exposure of human RPE cells, obtained by differentiation of iPSC, to serum from AMD patients mimics some of the early signs of the disease in vitro

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Age-related macular degeneration (AMD) is considered the main cause of legal blindness in the population over 50 years of age. The cells that firstly become affected in this disease and show signs of degeneration are those of the retinal pigment epithelium (RPE), whose proper functioning is fundamental for a healthy retina. These highly polarized cells, which form a monolayer, play an important role in maintaining the blood-retinal barrier and recycling the visual pigments of the photoreceptors, among other functions. The correct positioning of polarity proteins in the cells, such as the CRB proteins in the apical side and SCRIBBLE in the basolateral side, is essential for the performance of the RPE. In this work, we have developed a pathologic AMD-like iPSC-based RPE in vitro model. For this, human iPSC cells were cultured in 6-well plates until they achieved a stage of immature RPE. At this point, they were transferred to Transwell® inserts until they formed a fully matured and polarized epithelial monolayer. Then, RPE cells were exposed to blood serum obtained from patients diagnosed with dry or wet AMD and from control patients. Samples from these cultures, plus cells not exposed to blood serum, were collected 3 and 7 days after the treatment, and they were analyzed by immunofluorescence and western blot to assess the influence of the human serum on the integrity of the RPE cell monolayer. The analyses revealed that the distribution and organization of the polarity proteins and cell junctional proteins are altered under these in vitro simulated pathologic conditions. The mislocation of these proteins will prevent the RPE from fulfilling its key functions. Finally, these observations indicate that this model could be useful as an in vitro AMD model, allowing to analyze the alterations that the RPE undergoes in the onset and progression of this pathology.

Extraparenchymal granules in the fissura magna and hippocampal PAS Granules in APP/PS1 mice

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Polyglucosan aggregates known as Periodic Acid-Schiff (PAS) granules progressively accumulate in the mouse brain with aging. These granules, generated in astrocytes, contain carbohydrate-based neo-epitopes recognized by natural IgM autoantibodies (IgMs), increase with age and have been associated with brain clearance mechanisms. Apart from hippocampal PAS granules, we have recently described extraparenchymal (EP) IgM-reactive granules in specific brain fissures, particularly in the fissura magna, as well as IgM+ astrocytes near the glia limitans. These structures may also play a role in brain immune surveillance as well as with brain clearance and the glymphatic system.

In this study, we quantify hippocampal PAS granules in brain sections of APP/PS1 mice, a widely used Alzheimer's disease (AD) model at different ages and in control, age-matched C57BL/6J mice. We also analyse the presence, quantity, and distribution of EP granules and IgM+ astrocytes, using IgM to detect neo-epitope-containing structures and GFAP to label astrocytes.

Understanding the divergences in these IgM-reactive structures in APP/PS1 mice respect to the control age-matched mice will provide new insights into their role in neurodegeneration and brain clearance pathways.

Failure of Oligodendrogliogenesis in the Subventricular Zone during the Early Onset of Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by profound episodic memory loss. Recent evidence points to demyelinating pathology as an early event in the disease, indicating that myelinating alterations may precede tau and Aβ pathologies and even before white and grey matter atrophy.
As a source of oligodendrocytes in adulthood we find the neurogenic and oligodendrogenic niche of the ventricular-subventricular zone (V-SVZ). This zone generates neural stem cells that in turn give rise to transit-amplifying progenitors, which can differentiate into mature neuroblasts, astrocytes or oligodendrocytes. A key microdomain for oligodendrogliogenesis was identified in the dorsal V-SVZ, where the transcription factor Olig2 regulates the generation of oligodendrocytes that migrate to the corpus callosum and white matter tracts of the striatum and the fimbria fornix. In the region of the fornix, the demyelinating process in AD has been described, and this area of connection between the two hippocampi is closely involved in recognition memory.
This work aims to evaluate abnormalities in the SVZ and their impact on the oligodendroglial lineage, in young transgenic APP/PS1 mice model, at the age of 6 months, to analyse the proliferation and senescence of precursor cells in this region in line with a failed remyelination process in AD.
In the AD mouse model compared to controls. The senescent state of these cells could affect the generation and supply of mature myelinating oligodendrocytes in key areas such as the fornix, contributing to the memory defects shown in these animals.
In conclusion, the accumulation of senescent cells in the dorsal SVZ could be an underlying mechanism of the oligodendroglial and remyelinating dysfunction observed in AD. This study reinforces the importance of the integrity of neural stem cells and their progeny in the V-SVZ during early AD progression, highlighting the need to investigate therapies targeting neurogenesis and remyelination.

FOXP2 regulates positive- and cognitive-like symptoms associated with schizophrenia

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Schizophrenia is a neuropsychiatric syndrome that affects around 1% of the world population. It is characterized by positive symptoms, negative symptoms and cognitive deficits. Antipsychotic treatments can manage psychotic symptoms in acute episodes. However, their side effects and limited benefits on sociability and cognition can lead to poor medication adherence and increased risk of relapse. Finding new therapeutic targets that could alleviate this myriad of symptoms represents an unmet medical need.

Specific gene variants in the forkhead-box P2 (FOXP2) gene have been linked to a higher risk of developing schizophrenia. However, some of these associations are still controversial and it remains to be confirmed whether this transcription factor could play an active role in the disorder.

In our lab, we aim to decipher the contribution of FOXP2 to the pathophysiology of schizophrenia. By immunoblot analysis, we found aberrant expression of FOXP2 in the hippocampus of schizophrenia patients as well as in a ketamine mouse model of experimental psychosis. Manipulation of Foxp2 levels in the hippocampus of wild-type mice to mimic human condition using adeno-associated viral vectors provoked some positive-like symptoms of the disease, inducing an increase in the total distance travelled and changes in navigation in the open field test. However, no effects in the negative-like symptoms were found in the three-chamber sociability test. Interestingly, Foxp2 manipulation induced cognitive deficits related to an impairment in recognition memory in the Novel Object Recognition test, while we did not find any differences in the spatial short-term memory when performing the T-maze test. Ongoing proteomic analysis from the hippocampus of these mice will allow us to define potential candidates and molecular mechanisms underlying these effects.

In summary, our results suggest that aberrant levels of FOXP2 in the hippocampus of patients with schizophrenia can contribute to the positive and cognitive symptoms of the disorder.

Functional aspects of extracellular vesicles in Alzheimer's disease pathology

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Background:
This study examines the role of extracellular vesicles (EVs) in sporadic Alzheimer's disease (sAD). EVs transport RNA and proteins, including prion protein (PrP), which may mitigate amyloid toxicity.
Methods:
EVs from sAD and non-demented patients were isolated using ultracentrifugation and a density gradient. Purity was assessed via NTA, TEM, western blotting, and proteomic analysis. EV and PrP presence in amyloid (Aβ) plaques was examined through immunostaining and biochemical isolation. iPSC-derived cortical neurons from sAD and control patients were cultured and treated with sAD-EVs. sAD-like phenotypes and EVs from these cultures were analyzed using ELISA, western blotting, mRNA sequencing, and microscopy.
Results:
NTA and TEM analysis showed no morphological differences in the sAD-EVs. The expression of sAD hallmarks (p-Tau and Aβ) in the EVs was validated. The samples expressed EV markers and sAD-relevant proteins, showing an increased PrP expression in the late stage of the pathology. The proteomic analysis showed interesting differentially expressed proteins in the sAD-EVs that may drive their role in the pathology. The presence of PrP and EVs was confirmed in the amyloid plaques.
The iPSC-derived neuronal model showed cortical neuron fate and maturity and an sAD-like phenotype. The results obtained from treating them with sAD-EVs are promising, but further analysis is needed.
Conclusion:
Here, we show that the sAD-EVs can describe the pathological state of the brain-affected region. Moreover, we validated the expression of PrP in the EVs and amyloid plaques, suggesting that EV-PrP plays an important role in mediating the Aβ aggregation. The iPSC model proved useful for studying BDEVs in disease progression, warranting further investigation.

Functional electrical stimulation enhances the effects of locomotor rehabilitation in spinal cord injury rats

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Sensorimotor rehabilitation remains the most widely applied and effective intervention to enhance functional recovery in individuals with spinal cord injury (SCI). Although the activity-dependent plasticity mechanisms induced in spinal networks are not fully understood, extensive research in both human and animal models has demonstrated the critical role of intrinsic rehabilitation variables in maximizing recovery. However, given that the achieved functional gains are often incomplete, alternative or complementary interventions are being explored to enhance sensorimotor outcomes. This study aims to evaluate the locomotor recovery and plastic changes in SCI rats subjected to bilateral alternating functional electrical stimulation (FES) of the hindlimb extensor muscles while awake but not performing any exercise. Their recovery was compared to that of animals undergoing daily treadmill training. After two months of treatment, both groups exhibited significant improvements in sensory and motor function compared to untreated injured animals, as evidenced by a two-point increase in the BBB scale. However, no significant behavioral or electrophysiological differences were observed between the two rehabilitated groups.
We conclude the feasibility of FES to promote spinal plasticity and motor recovery and its utility to substitute or add to conventional task specific rehabilitation.

Functional, morphological, and transcriptomic characterization of fused Purkinje cells in a multiple sclerosis mouse model

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Neurodegenerative diseases are increasing, and effective treatments are scarce. Among the emerging therapeutic strategies, cell therapy is one of the most promising. Bone marrow-derived cells (BMDCs) can migrate into the brain, where they transdifferentiate into different cell types or fuse with neurons, such as Purkinje cells. Although fusion events are rare, their frequency increases in certain pathologies, including multiple sclerosis. In the Experimental Autoimmune Encephalomyelitis (EAE) model of multiple sclerosis, fused cells remain functional and retain two transcriptionally independent nuclei, suggesting that BMDCs could be used as vehicles for delivering therapeutic agents. However, mechanisms underlying cell fusion and properties of fused cells remain poorly understood.

To investigate cell fusion events in EAE mice, we developed a protocol involving the transplantation of GFP-positive BMDCs to identify fused cells. We optimized the timing between transplantation, EAE induction, and tissue collection to detect fusion events at the earliest possible stage. Using Patch-Seq technology, we compared individual fused and nearby non-fused Purkinje cells. We recorded cells' electrophysiological activity, stained them for morphological analysis, and collected their cytoplasm for single-cell RNA sequencing. We complemented these studies by analysing transcriptional activity and the epigenetic state of the two nuclei of fused cells.

Our preliminary results show that the morphology of fused and non-fused cells is quite similar, while action potential frequency is lower in fused cells. Fused cells also express typical BMDC genes that are not expressed in non-fused Purkinje cells.

These results will help us understand the nature of cells involved in fusion and its consequences, bringing us closer to using BMDCs as therapy for neurodegenerative diseases.

Support: Science, Innovation and Universities Spanish Ministry (PID2022-140525NB-I00, PID2022-140456NB-I00, FPU2020); JCyL-FEDER (SA112P24); FENS/IBRO-PERC Exchange Fellowship; Erasmus+ programme Partnership for Cooperation (2023-1-PL01-KA220-HED-000160284); Strategic research programmes of excellence from JCyL, co-funded by the ERDF Operational Programme (CLU-2023-1-01).

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Galectin-3 Suppression Enhances Purkinje Cell Dendritic Arborization and Delays Motor Coordination Deficits in the tambaleante Ataxia Mouse Mode

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The autosomal recessive Gly483Glu mutation in the murine Herc1 protein induces cerebellar overexpression in tambaleante mice (tbl), which leads to a cascade of molecular disruptions including the dysregulation of autophagy-mediated Purkinje cell (PC) death and alterations in the PI3K/AKT/mTOR signalling pathway, ultimately impairing axonal myelination. These disruptions contribute to the development of an ataxic syndrome characterized by profound motor deficits. Notably, prior to the degeneration of PCs, cerebellar microglia adopt a neurodegenerative phenotype (MGnD), marked by the overexpression of Galectin-3 (Gal3), a key player in the neuroinflammatory response.
To better understand the role of microglia in PC degeneration, we generated a tbl/Gal3KO model lacking the Lgals3 gene (Gal3). Despite no significant differences in microglial numbers between tbl and tbl/Gal3KO mice, rotarod performance tests revealed substantial improvements in motor coordination in 2-month-old tbl/Gal3KO mice. Immunohistochemical analysis further showed partial recovery of the PC population and enhanced dendritic arborization complexity in surviving PCs within the tbl/Gal3KO group. These findings suggest that the ablation of Gal3 mitigates tbl pathology through two complementary mechanisms: cellular rescue, which helps preserve PC viability, and supports dendritic remodelling, partially restoring dendritic arborization patterns. This study highlights the critical role of Gal3 as a modulator of both Purkinje cell survival and dendritic integrity in the context of cerebellar ataxias, operating independently of microglial proliferation.

Glial Cell Activation, Neuroinflammation and Neurodegeneration in the Cerebellum of a Mouse Model of Friedreich's Ataxia

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Friedreich ataxia is a hereditary disorder resulting from reduced levels of the protein frataxin (FXN) due to an expanded GAA repeat in the FXN gene. This deficiency causes several neuropathological defects including a progressive cerebellar dysfunction and atrophy which results in the loss of movement coordination and equilibrium.
In this work, we followed and characterized the progression of changes in the cerebellar cortex of the latest version of Friedreich ataxia humanized mouse model, YG8-800 (Fxnnull:YG8s(GAA)>800), which carries a human FXN transgene containing >800 GAA repeats. These YG8-800 mice exhibit an ataxic phenotype characterized by poor motor coordination, decreased body weight, cerebellar atrophy, neuronal loss, and changes in synaptic proteins.
Our study revealed that YG8-800 mice exhibit a progressive reduction in the main cerebellar neuronal population, the cerebellar granule neuronss, which correlates with progressive atrophy at the structural level and impairment of locomotor function at the functional level.
Interestingly, the earliest observed change in the cerebellum of these mice, preceding neurodegeneration, was the activation of glial cells, both astrocytes and microglia, which was accompanied by the increased expression of key proinflammatory cytokines and downregulation of neurotrophic factors.
The in-depth characterization of the YG8–800 mouse model allows the investigation of the pathogenic molecular mechanisms responsible for Friedreich's ataxia and represents a convenient experimental tool for preclinical evaluation of possible therapies.

HeliCure targets α-Synuclein oligomers and rescues motor deficits in a preclinical mouse model of Parkinson's disease

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Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the accumulation of pathological α-synuclein (αSyn) aggregates. While physiological αSyn exists as a functional monomer, its misfolding and aggregation into toxic oligomers and amyloid fibrils drives neurodegeneration. Current therapies alleviate symptoms but fail to target these disease-associated aggregates, underscoring the need for strategies that selectively neutralize pathological species. Here, we evaluate the efficacy of HeliCure, a peptide that selectively targets αSyn oligomers, in a PD mouse model with AAV2/5-induced overexpression of human wild-type αSyn (h-αSyn) in the substantia nigra pars compacta (SNc). Control group received an AAV2/5 empty vector. Six weeks after AAV2/5 infusion, HeliCure was administered intracerebroventricularly (ICV) or intranasally (IN) at 0.6-0.87 µg/day for 14 days. Motor function (open field, beam walking, cylinder, and rotarod tests) and gastrointestinal motility were assessed at 8 weeks after AAV2/5 infusion. H-α-Syn (monomeric and oligomeric species), as well as tyrosine hydroxylase (TH, limitant enzyme of dopamine- DA synthesis), microglial Iba-1, and astrocyte GFAP levels in the striatum and SNc were assessed by Western-Blot. Mice with overexpression of H-αSyn treated with HeliCure showed a significant reversal of motor deficits and a restoration of intestinal motility compared to the respective control group. Comparable results were detected after ICV and IN administration. Western-blot analysis revealed a reduction in monomeric and oligomeric h-αSyn levels in the striatum and SNc, alongside partial reversion of TH levels only in striatum. No changes were detected in Iba1 and GFAP levels in SNc and striatum in any of the groups examined. Our findings demonstrated HeliCure's potential to suppress αSyn aggregation and rescue functional deficits in a translationally relevant PD mouse model. The efficacy of non-invasive intranasal delivery highlights its clinical potential as a disease-modifying therapy.

Hierarchy and Criticality in Post-Stroke Neural Dynamics: Insights from Intrinsic Neural Timescales

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Stroke severely disrupts brain function, yet its effects on temporal dynamics—crucial for information processing and recovery—remain unclear. Intrinsic neural timescales (INT), which measure the temporal persistence of neural activity, provide a framework for studying these changes. However, the extent and mechanisms of stroke-induced INT alterations are not well understood.

This study analyzed longitudinal resting-state fMRI data from 15 ischemic stroke patients, scanned five times over six months, comparing their INT values with age-matched healthy controls. INT was calculated from the area under the positive autocorrelation function of BOLD signal fluctuations. We examined INT's hierarchical organization across brain networks and its relationship with motor recovery, grouping patients into good and poor recovery categories. Additionally, using the Kinouchi & Copelli model, we explored how neural excitability influences INT within a criticality framework.

Results showed stroke patients had significantly elevated INT, persisting across recovery stages. Early post-stroke, INT's hierarchical structure was disrupted. By two months, poor recovery patients exhibited prolonged INT in the dorsal attention, language, and salience networks. These findings align with excitability models, suggesting stroke shifts brain dynamics toward criticality or even a supercritical state, where excessive signal persistence hinders processing.

Our results indicate that stroke-induced INT prolongation reflects increased neural network excitability, pushing the brain toward criticality or even into a supercritical state. Persistent INT abnormalities in poorly recovering patients suggest INT as a prognostic biomarker for long-term outcomes. This study offers new insights into stroke-induced disruptions in brain criticality and highlights non-invasive neuromodulation as a potential strategy to restore normal INT and improve recovery.

HTT intermediate alleles and microRNA dysregulation in LOAD: a link to spliceosome dysfunction and Tau pathology

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Late-onset Alzheimer's disease (LOAD) accounts for over 95% of AD cases and more than 7% of LOAD patients present intermediate alleles in the huntingtin gene (HTT IAs). We have previously analyzed a clinical and neuropathological cohort of LOAD patients carrying HTT IAs, LOAD non-carriers and healthy controls. In this cohort, LOAD carriers of HTT IAs had reduced survival after disease onset, increased Tau 3R aggregation, and more HTT-positive neurons in the caudate nucleus, particularly vulnerable to these expansions. Thus, we hypothesize that HTT IAs act as copathological trigger, inducing epigenetic changes through microRNAs (miRNAs) and modifying critical gene expression. Altered miRNA pattern can further contribute to accelerated disease onset and/or progression in LOAD patients carrying HTT IAs.
Postmortem caudate nucleus tissue from LOAD carriers (n=14), LOAD non-carriers (n=13), and healthy controls (n=5) underwent miRNA NGS, in silico analysis, and molecular/histopathological studies. MiRNA profiling in the caudate nucleus showed significant alterations in both LOAD groups vs. controls, being more pronounced in LOAD carriers. In silico analysis indicated that differentially expressed miRNAs regulate key neurodegenerative processes, highlighting the spliceosome pathway, mediated by SRSF family splicing factors and the nuclear FUS-SFPQ complex. In fact, both FUS and SFPQ were increased in the caudate nucleus, possibly contributing to the previously described imbalance in Tau isoform processing. Moreover, LOAD patients carrying HTT IAs showed an accelerated progression of neurofibrillary tangles aggregation, with a significant increase in ghost tangles (the last state of Tau aggregation), supporting a more advanced pathology in these patients. Our findings evidence a synergistic effect of HTT IAs and miRNA dysregulation, driving accelerated LOAD progression, as evidenced by neuropathological changes. Genetic screening for HTT IAs may enhance patient stratification, facilitating tailored clinical management and personalized care.

Human adult hippocampal neurogenesis is shaped by neuropsychiatric disorders, demographics, and lifestyle

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The mammalian hippocampus is one of the few regions of the brain to host the addition of new neurons during adulthood, a phenomenon known as adult hippocampal neurogenesis (AHN). As a result, newly formed dentate granule cells incorporate into hippocampal circuits, modulating hippocampal-dependent functions. Patients with neuropsychiatric disorders show structural and functional abnormalities in the hippocampus. However, whether AHN impairments underlie these alterations remains unknown. To address this question, we aimed to determine whether major depression (MD), schizophrenia (SCH), and bipolar disorder (BD) compromise the integrity of human AHN and the homeostasis of the dentate gyrus neurogenic niche – a specialized micro-environment in which new neurons grow. With this objective, our study examined a cohort of 59 human subjects (24 females and 35 males). Coded fresh frozen glass-slide-mounted 14µm hippocampal sections were obtained, including tissue from 14 neurologically healthy control subjects and 45 patients (15 per diagnosis group: MD, SCH, and BD). Using immunohistochemistry and confocal microscopy imaging, we conducted an in-depth analysis of hippocampal sections to assess alterations across individual AHN stages in neuropsychiatric disorders. Additionally, we evaluated alterations of the neurogenic niche components including astrocytes, microglia, and blood vessels. Morphometric characterizations and unbiased stereological cell quantifications were used to characterize distinct cell populations. Demographic and lifestyle data were also included in our analysis. Our findings demonstrate that human AHN is shaped by distinct neuropsychiatric disorders and that it is further influenced by demographic and lifestyle factors. These data might be relevant for the design of future therapeutic strategies to prevent or treat mental health disorders.

Human and mouse Alzheimer's seeds differentially affect amyloid and tau aggregates in aged mice

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Alzheimer's disease (AD) is a complex neurodegenerative proteinopathy in which Aβ and tau misfold and aggregate into entities that structurally unsettle native proteins, mimicking a prion-like or "seeding" process. These Aβ and tau "seeds" can arrange in different conformations or strains that might display distinct pathogenic properties. Furthermore, recent evidence suggest that microglia play a key role in the amyloidogenic event and can modulate the propagation and aggregation processes. Here, we investigated whether amyloid seeds from human AD brains compared to those from transgenic mice (3xTg-AD) are more prone to induce Aβ and tau aggregates in vivo, as well as potential differences in the microglial response to the plaque pathology. We employed histological and molecular approaches to determine the Aβ/tau pathology and Aβ-seeding capacity of brain extracts derived from postmortem AD cortex versus aged 3xTg-AD mice (25-month-old). Brain homogenates were injected into the hippocampus of 3xTg-AD mice and examined at 17-18 months of age. The seeds from the human AD brain induced more aggressive amyloid pathology compared to seeds from aged 3xTg-AD mice. However, the AD seeds from aged transgenic mice triggered more tau pathology. Interestingly, such mice seeds impaired microglial clustering around plaques leading to more severe neuritic pathology. These results suggest that multiple variables such as the AD seed, recipient model and time, are critical factors that can modulate the amyloid pathology onset and progression. Thus, more profound understanding on these factors will provide key insight on how amyloid pathology progresses in AD.

Human Dental Pulp Stem Cells enhance brain neovascularization and neurological recovery following intracarotid injection in ischemic stroke

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Ischemic stroke results from reduced cerebral blood flow (CBF), leading to neuronal death due to oxygen and nutrient deprivation. Current therapeutic strategies focus on restoring CBF through thrombolysis, but additional approaches, such as stem cell therapy, could enhance neuroprotection and vascular regeneration. Human Dental Pulp Stem Cells (hDPSCs) are a highly accessible stem cell source with notable immunomodulatory and neurotrophic properties. They can differentiate into vascular cells and generate functional blood vessels, making them promising candidates for neurovascular repair. Previous studies demonstrated that hDPSCs contribute to neovasculogenesis in the murine brain, but their potential in ischemic stroke remains unexplored.
This study investigated whether intracarotidally injected hDPSCs could reach ischemic brain regions and promote neovascularization in Sprague-Dawley rats subjected to 75 minutes of middle cerebral artery occlusion (MCAO). hDPSCs, isolated from third molars of healthy donors, were cultured in serum-free media to promote vascular differentiation before injection one hour post-occlusion. Neurological test recovery was periodically assessed, and animals were sacrificed at 14 days post-MCAO for histological analysis. Immunostaining was performed for human-specific markers (STEM121, hCD31), neural progenitor markers (DCX, Ki67, Tuj1), and vascular integrity markers (lectin, GFAP). Grafted hDPSCs were detected within the infarct core and peri-infarct regions, where they contributed to neovascularization, as evidenced by the formation of hCD31+ blood vessels surrounded by GFAP+ astrocytes. These vessels exhibited well-formed basal laminae, suggesting blood-brain barrier restoration. Additionally, a higher migration of neural progenitors towards the infarct area was observed. No tumor formation was detected in liver or spleen sections. Neurological assessments indicated improved recovery in hDPSC-grafted animals compared to controls.
Future experiments will assess blood-brain barrier permeability using Evans Blue at different time points and evaluate infarct volume through cresyl violet staining. These will provide additional insights into the therapeutic potential of hDPSCs for ischemic stroke.

Human Induced Pluripotent Stem Cell-Derived Astrocytes From Alzheimer´s Disease Patients Show A Senescent Phenotype Which Compromise Their Functionality

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Background:
Alzheimer's disease (AD) is characterized by a complex pathology, not fully resolved yet. This fact, together with the lack of reliable models, has impeded the development of effective therapies. Glial cell dysfunction has been proposed to be involved in AD pathogenesis, but this cannot be properly modeled using the available animal models, so we hypothesized that cells derived from AD patients can serve as a better platform for studying the disease. In this sense, human pluripotent stem cells (hPSC) allow the generation of different types of neural cells, which can be used for disease modeling, identification of new targets and drugs development.

Methods:
We have generated hiPSC-derived astrocytes from AD patients and cognitively unimpaired age-matched individuals and evaluated their metabolism and phenotype employing confocal imaging, immunofluorescence, flow cytometry, RT-qPCR and functional assays.

Results:
All astrocytes expressed functional markers including aldehyde dehydrogenase 1A1 (ALDH1A1), glutamate transporter GLAST, glial fibrillary acidic protein (GFAP), aquaporin-4 (AQP4) and vimentin. However, astrocytes from AD patients showed increased expression of reactive markers. In addition, these astrocytes derived from AD patients showed significant metabolic alterations associated with a pro-inflammatory and senescent phenotype which in turn impair their neuronal support as measured in coculture assays.

Conclusions:
Our preliminary data suggest that astrocytes derived from AD patients present an intrinsic pro-inflammatory and senescent phenotype which compromise their functionality. Elucidating the mechanisms inducing these processes and their functional consequences should help for a better understanding of role that astrocytes play in AD, by direct functioning and also through their interactions with neurons and the other glial cells. This should lead to the identification of potential therapeutic targets for future AD treatments.

Supported by ISCiii, grants PI21/00915 and PI24/00274 (AG) and PI21/00914 and PI24/00308 (JV), Ciberned (PI2022/01, AG and JV), Sumaira Foundation TSF_SPARK_2023_03 (JAGL) and IBIMA INN24_02 (to JAGL).

ID-linked KDM1A and KDM5C cooperate to maintain enhancer homeostasis and suppress spurious transcription safeguarding neuronal function in mature excitatory neurons

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Lysine demethylases (KDMs) play a crucial role in determining cell fate, and their loss or reduction has been linked to intellectual disability (ID) and neurodevelopmental disorders. KDM1A and KDM5C are two of the most highly expressed KDMs in hippocampal neurons. These two enzymes converge in maintaining a repressive state, and the ablation of either one of them in adult hippocampal excitatory neurons triggered the derepression of non-neuronal genes in neurons. Given the phenotypic similarities between Kdm1a- and Kdm5c-deficient mice, along with the shared role of both enzymes in maintaining a repressive state through histone H3 at lysine 4 (H3K4) demethylation, we explored their functional interaction by generating and characterizing double inducible, forebrain-specific knockout mice (dKDM-ifKOs). We observed that KDM1A and KDM5C share many genomic targets, and their combined depletion exhibited exacerbated ectopic transcription and much broader epigenetic dysregulation compared to single ifKOs, leading to the appearance of numerous H3K4me3 de novo peaks that promote spurious transcription in intronic and intergenic regions, along with the disorganization of H3K27me3 repressive domains. At the phenotypic level, the combined ablation of KDM1A and KDM5C also caused more severe behavioral alterations than the individual loss of either enzyme. Neurological defects were accompanied by altered hippocampal expression of ionic channels and increased excitability of hippocampal CA1 pyramidal neurons. These results underscore the convergent role of both ID-linked KDMs in regulating cell-type specific gene silencing and fine-tuning H3K4 methylation levels at promoters and enhancers, safeguarding chromatin organization and the intrinsic properties of mature neurons, and therefore cognitive functions in adulthood.

IGF-II: a neuroprotective agent against Cocaine-induced oxidative stress and apoptosis

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Excessive cocaine (benzoylmethylecgonine) consumption has been associated with neurodegenerative diseases affecting dopaminergic neurons, such as Parkinson's disease (PD). Insulin-like growth factor II (IGF-II) has demonstrated protective effects against oxidative stress induced by neuropathologies and drug exposure. This study evaluates the impact of cocaine on neuronal morphology and viability and investigates IGF-II's potential neuroprotective role using SN4741 dopaminergic cells.

Cells were exposed for 24 hours to either cocaine alone or cocaine with IGF-II. Cell death (LDH assay) and morphological alterations (Giemsa staining) were assessed, alongside mitochondrial integrity and function (OCR, Seahorse), membrane potential (JC-1), and ROS production (DHE) after 6-hour treatments.

An optimal cocaine concentration (2 mM) was measured, inducing 39.75% neuronal death. IGF-II (1.5 ng/mL), established as effective in previous studies, significantly reduced cocaine-induced cell death (280% increase vs. control). Morphological changes induced by cocaine, including globular shape, membrane thinning, loss of neurites, and cell aggregation, were prevented by IGF-II co-treatment. Cocaine significantly increased ROS levels (173.6%) and reduced mitochondrial membrane potential (20%), both indicative of apoptosis induction. IGF-II effectively reversed these effects, preventing oxidative stress and preserving mitochondrial function. Furthermore, IGF-II mitigated a 60% reduction in mitochondrial oxygen consumption induced by cocaine.

To conclude, IGF-II exhibits potent antioxidant and neuroprotective properties, counteracting cocaine-induced oxidative stress, mitochondrial dysfunction, and neuronal degeneration. These findings support IGF-II as a potential therapeutic agent against cocaine-related neurotoxicity.

Impact of clinical severity on the thalamic neuroinflammatory patterns in experimental multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated, inflammatory, and demyelinating disease of the central nervous system. Due to its heterogeneity in clinical severity and treatment response, biomarker identification is essential for monitoring disease progression. The thalamus is a crucial structure severely impacted in MS patients, where ongoing demyelination, inflammation, and neurodegeneration lead to significant atrophy. Thalamic damage at early stages of MS has been associated to the future disease severity, being especially prominent in the progressive forms of the disease.

This study proposes a histopathological analysis of the ventral posterolateral (VPL) and lateral geniculate (LGN) thalamic nuclei to explore whether disease severity is associated with the degree of demyelination, resident and peripheral immune cell activity, and neuronal density in the experimental autoimmune encephalomyelitis (EAE) MS model. Additionally, we investigate monocytic myeloid-derived suppressor cells (M-MDSCs) as potential biomarkers of thalamic alteration. Tissue was obtained at the time of maximum clinical severity (peak) and the histopathological quantification performed in a disease severity individualized manner.

We observed that severe EAE is associated to greater astrogliosis, microgliosis, and increased density of glutamatergic terminals in both thalamic nuclei. Demyelination is confined to the VPL, regardless of EAE severity, with no evidence of neurodegeneration in either nucleus. The M-MDSC levels at onset were exclusively associated with increased Arg-I+ infiltrates in the LGN. Moreover, a direct correlation between Arg-I+ and CD3+ T cells was observed in both nuclei. Interestingly, circulating M-MDSCs at the peak inversely correlated with VGlut2 density, and splenic M-MDSCs were associated with less prominent astrogliosis in both nuclei and reduced microglial activation in the LGN.

In sum, EAE severity leads to nucleus-specific effects on the thalamus. While M-MDSCs failed to be a reliable biomarker for thalamic impairment at disease peak, their potential role in neurodegeneration, particularly in chronic stages of the disease, warrants further investigation.

Impact of inflammation on myelin endocytosis and degradation by macrophages in vitro

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Traumatic central nervous system (CNS) injuries result in cell death, leading to the release of myelin and other cellular debris. The accumulation of myelin at the injury site triggers inflammation, is cytotoxic, and hampers axonal regeneration. The clearance of myelin debris depends on endocytosis followed by metabolic breakdown by microglia and macrophages from the bloodstream. However, these processes are often impaired by the inflammatory environment. Our objective was to investigate how inflammatory stimuli influence the uptake and degradation of myelin by bone marrow-derived macrophages (BMDM) and the macrophage cell lines Raw264.7 and J774.A1.

We used myelin labeled with pHrodo and fluoromyelin to study myelin uptake and degradation. Additionaly, we employed Lipi-Blue and BODIPY fluorophores to track the intracellular accumulation of lipid droplets. Quantitative measurements were made using a microplate reader, immunofluorescence microscopy and cytometry. At a low myelin concentration (20 µg/ml), neither LPS nor IFNγ appeared to affect myelin degradation in either cell type. However, increasing the myelin concentration (to 50 or 100 µg/ml) led to the accumulation of myelin in the cytoplasm of Raw264.7 cells. In contrast, both BMDM and J774.A1 exhibited increased myelin uptake, leading to lipid droplet accumulation within the cells.

Inflammatory conditions increased the accumulation of lipid droplets in Raw264.7 and J774.A1 cells, resembling foam cells observed in vivo. However, this effect of inflammation was not pronounced in BMDM.

In conclusion, both IFNγ and LPS interfere not only with the uptake of myelin by macrophages (Wu et al, 2022; Romero-Ramírez et al. 2024) but also with the subsequent intracellular recycling processes.

Impact of Treg expansion on Wallerian degeneration and remyelination after peripheral nerve injury

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Regulatory T cells (Tregs) are key immune modulators with roles in maintaining immune homeostasis and regulating tissue repair. Their functions in peripheral nerve regeneration remain poorly understood. This study investigates the role of Tregs in Wallerian degeneration, axonal regeneration, and remyelination following sciatic nerve injury in mice.

We used 8-week-old male and female mice subjected to a sciatic nerve crush injury. Treg infiltration in the distal nerve stump was assessed at 0, 7, 14, and 28-days post-injury using flow cytometry. To evaluate the impact of Treg expansion on nerve repair, endogenous Tregs were systemically expanded via co-injection of recombinant IL-2 and anti-IL-2 monoclonal antibodies. Ultrastructural analyses of axonal regeneration and remyelination were performed using electron microscopy, quantifying axon diameters, myelin thickness, and g-ratio values.

Tregs infiltrated the injured nerve, peaking at day 14 post-injury. Systemic Treg expansion significantly increased their numbers in peripheral and lymphoid tissues. However, structural analysis at 14 and 21-days post-injury revealed no significant differences in axonal regeneration or remyelination between Treg-expanded and control groups. Myelinated axon density, g-ratio values, and myelin thickness were comparable between conditions. No sex-dependent differences in Treg accumulation were detected.

Our findings indicate that while Tregs infiltrate the injured peripheral nerve and can be expanded systemically, their presence does not enhance axonal regeneration or remyelination. Further research is required to elucidate their role in modulating the inflammatory microenvironment and interactions with Schwann cells. Understanding these mechanisms could pave the way for novel therapeutic strategies targeting immune regulation in peripheral nerve repair.

Acknowledgment
This work was partially supported by a grant from the Spanish State Research Agency under the “Proyectos de Generación del Conocimiento 2022” program (PID2022-143269OB-I00), a grant from the Convocatoria Intramural 2023 (ISABIAL), and a Miguel Servet Fellowship from the Spanish Health Institute Carlos III awarded to JAGS (CP22/00078).

Impaired unfolded protein response, BDNF and synuclein markers in dorsolateral prefrontal cortex and caudate nucleus of patients with depression and Parkinson's disease

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Major depressive disorder (MDD) is characterized by significant impairment in social, emotional, and cognitive functioning. Its precise pathophysiology remains poorly understood. Alterations in protein homeostasis and some misfolded proteins have been identified within the brains of patients diagnosed with neuropsychiatric disorders. In contrast to neurodegenerative processes such as Parkinson's disease (PD), where the accumulation of aggregated α-synuclein (α-Syn) protein is a primary cause of significant neuronal loss, altered proteostasis in MDD may result in loss-of-function effects by modifying synaptic neuroplasticity. Moreover, aberrant activation of endoplasmic reticulum (ER) pathways may intensify the pathological alterations due to altered proteostasis. In this study, dorsolateral prefrontal cortex (dlPFC) and caudate nucleus from MDD patients and non-psychiatric controls were used. Postmortem samples of same brain areas from PD patients (Braak 2–3 and 5–6) and controls were also included. Protein levels of ER and unfolded protein response (UPR), synucleins (α-, β- and γ-Syn), and brain-derived neurotrophic factor (BDNF) were measured by Western-Blot. Phospho-eIF2α/eIF2α ratio was increased in the dlPFC and caudate nucleus of MDD and PD patients compared to their respective controls. Brain area-dependent changes in BiP and GRP94 levels were also found. We further detected accumulation of immature BDNF precursors and opposite changes in α- and β-Syn levels in the dlPFC of MDD and PD patients compared to controls. Our findings suggest that alterations in proteostasis contribute to the pathophysiology of MDD, as previously described in PD. A deeper understanding of the pathways involved will identify other candidate proteins and new targets with therapeutic potential.

Financial support: Grant PID2022-141700OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and "ERDF A way of making Europe"; AGAUR_SGR21-01358 Generalitat de Catalunya.

In vivo reprogramming to rejuvenate aged tissues induced by chronic stress

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Major Depressive Disorder (MDD) is a severe psychiatric condition characterized by persistent sadness, anhedonia, and occasional suicidal thoughts. It involves genetic and environmental factors, with stress being a major trigger. Chronic stress has been linked to accelerated cellular aging, leading to the exploration of different genetic or chemical approaches for tissue rejuvenation. However, such type of applications in the central nervous system for mitigating psychiatric disorders remains unexplored.

This study assessed the effect of tissue rejuvenation by in vivo genetic reprogramming on stress-induced behavioral, cognitive, and neurobiological changes in male and female mice. Reprogrammed females showed partial recovery from anhedonia (splash test), reduced anxiety (Open Field), improved object recognition (NORT), and decreased freezing in Fear Conditioning (FC). Intriguingly, males showed no significant behavioral improvements.

Histological analyses of the dentate gyrus revealed sex-specific effects. In males, reprogramming normalized stress-induced microglial circularity and reduced astroglial GFAP intensity. Sirt1 and H3K9Ac levels partially recovered. In females, H3K9me3 levels and stress-impaired cell migration (DCX marker) were restored. Sox2-related pluripotency remained unchanged. Moreover, neither chronic stress nor reprogramming affected the shape, size, branching complexity, or connectivity parameters of granular neurons in the dentate gyrus.

These findings underscore sex-specific responses to chronic stress and suggest that tissue rejuvenation through in vivo reprogramming may mitigate some behavioral and neurobiological effects. This study provides novel insights into the potential of these type of strategies for addressing stress-related psychiatric disorders.

Injury beyond the ischemic lesion core: distant BBB damage in a mouse model of stroke

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Stroke is a neurovascular pathology that affects nearly 15 million people each year, representing one of the leading causes of disability and mortality worldwide. Despite significant research progress, the mechanisms of brain damage following stroke are only partially understood. One of its major consequences is the disruption of the blood-brain barrier (BBB), which can expand beyond the ischemic core, affecting distant vascular territories with unknown consequences. The factors driving this distant BBB opening, as well as its role in secondary injury or tissue repair, remain unclear.
To investigate this phenomenon, we implemented a mouse model of thromboembolic stroke targeting the M2 segment of the Middle Cerebral Artery (MCA). Magnetic Resonance Imaging (MRI) was used to assess the size and location of the ischemic lesion, while BBB permeability was evaluated using the fluorescent tracer Cadaverine. A combination of 2D and 3D histological approaches was applied: iDISCO+ tissue clearing, 3D light-sheet microscopy, and bioinformatics tools (IMARIS and ClearMap) enabled brain-wide mapping of Cadaverine distribution, while classic 2D histology was used to analyze local cellular mechanisms in permeable distant regions. Our results reveal that BBB disruption extends far beyond the core lesion detected by T2-MRI, more often affecting specific vulnerable brain areas located in distant vascular territories. Understanding this widespread, MRI-silent consequence of focal ischemia will open new avenues for designing therapeutic strategies aimed at limiting injury expansion and diffuse damage in stroke.

Innovative cellular approach to delay disease onset and reduce neuroinflammation in an ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron loss, neuromuscular impairment, and respiratory failure, leading to death typically within 3–5 years of diagnosis. With a global prevalence of 4.42 cases per 100,000 people, no effective therapies currently exist. Its multifactorial nature limits the efficacy of treatments which target only one specific ALS-related feature. In this context, stem cell therapy has emerged as a promising approach due to its pleiotropic effects. While preclinical models have shown potential benefits of stem cell therapy, clinical trials face challenges in proving efficacy.

Here, we evaluated the therapeutic potential of an alternative, untested cell-based therapy in a SOD1-G93A ALS mouse model. Cells were administered repeatedly via a non-invasive route during the asymptomatic stage (8–11 weeks). Disease progression was monitored using the NeuroScore test. Mice were euthanized in short-term (12 weeks of age) or long-term (16 weeks of age) for histological and molecular analyses.

Our results showed that cellular treatment delayed the onset of ALS symptoms, particularly hindlimb paralysis. Plasma analysis evidenced a reduced systemic inflammatory state in treated mice. Cortical neuron preservation was observed in the motor cortex, in the short term, while in the lumbar spinal cord, motor neurons were protected in the long term alongside reduced muscle atrophy in the tibialis anterior muscle. RNA sequencing revealed that cellular treatment modulated inflammatory responses in the brain cortex, while affecting both inflammation and muscle-related pathways in the spinal cord.

Our findings support the potential of an innovative cellular multi-targeted strategy to provide neuroprotective benefits in ALS mice, delaying symptom onset and improving muscle condition. This study highlights the potential for disease-modifying effects and emphasizes the importance of further investigation into cellular therapies for ALS.

INVOLVEMENT OF DIFFERENT PLAQUE-ASSOCIATED MYELOID POPULATIONS IN THE BRAIN OF ALZHEIMER'S PATIENTS

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Microglia are the resident innate immune myeloid cells of the brain and play a critical role in the pathological process of Alzheimer´s disease (AD) since genetic risk variants for late-onset AD are expressed exclusively or highly in these glial cells. Though the diversity of functional states of microglial cells in AD continuum is still unknown, loss of microglial neuroprotective and phagocytic function indicates the critical involvement of malfunctioning microglia in driving pathological progression and neurodegeneration. Moreover, little is known about the diversity of myeloid cells across different brain regions along the AD continuum. In this work, we have analyzed the responsive myeloid phenotype to amyloid pathology in two AD vulnerable brain regions, frontal cortex and hippocampus. For this purpose, immunolabeling for a wide range of markers combined with image analysis approaches have been carried out in postmortem samples from AD patients with dementia (Braak V-VI) and age-matched asymptomatic cases (Braak II). While the frontal cortex showed strong microglial activation around plaques, the hippocampus of the same individuals showed an exhausted microglial response including degenerative/senescent features. Regional differences were also found in the microglial phagocytic capacity. By counting the number of different microglial subsets, according to the combination of markers expressed, we found that the microglial composition of plaques was highly heterogeneous. Interestingly, even though some Braak II individuals presented high amyloid pathology, only AD patients exhibited infiltration of CD163 monocyte-derived cells that invaded plaque near blood vessels. These results reveal the co-existence of distinct myeloid populations associated with amyloid plaques during disease progression and open the opportunity to design targeted therapies, not only to microglia, but also to peripheral immune cell population to modulate amyloid pathology.

Supported by ISCiii grants PI24/00274 (to AG) and PI24/000308 (to JV) co-financed by FEDER funds from European Union; and by CIBERNED

Is IKZF3 a new theranostic marker for Schizophrenia?

Is IKZF3 a new theranostic marker for Schizophrenia?

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IKZF3, a.k.a. Aiolos is a member of the Ikaros zinc finger protein (Ikzf) family. Aiolos together with other members of the Ikzf family are known to be essential for proper immune system development and function. Besides, some of the other members of the family, but not Aiolos, are known to be necessary for neural cells development and function and have been associated with psychiatric conditions such as schizophrenia. However, as far as we know, the potential implication of Aiolos in these contexts is still yet to be studied. Preliminary data of our lab indicates that the levels of Aiolos varies through development in several brain areas in mice. On the other hand, we have found dysregulations of Aiolos in immune and neural cells of schizophrenia patients. Overall, our results suggest that Aiolos is implicated in both contexts, brain development and schizophrenia neuro-immune pathology. As futures perspectives, we plan to deeper characterize the effect of Aiolos modulation in the brain of mice models. We hypothesize that the alteration of Aiolos will negatively impact on brain development and/or function. Likewise, we plan to evaluate possible alterations of Aiolos in other psychiatric conditions such as major depression disorder. We hypothesize that Aiolos is mainly dysregulated in schizophrenia and probably also in other psychiatric conditions.

KIF21B regulates microglial function in a sex-dependent and context-specific manner

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The cytoskeleton plays a crucial role in microglial functions, but its regulation during neuroinflammation remains poorly understood. Kinesin-family motor proteins mediate vesicular transport and cytoskeletal organization, yet the role of KIF21B in microglia has not been characterized.
We investigated KIF21B expression and function in microglia in an in vitro model. Our studies were performed in primary cultures of microglia from the cortex of postnatal sexed mice. To evaluate the role of KIF21B, we used a pool of 4 siRNAs to silence KIF21B and a non-target siRNA as a control. Phagocytosis assays were performed by seeding two substrates to cells: synthetic spheres covered with E. coli that increase their fluorescence in an acidic environment, and neuronal debris conjugated with Cy3. Cell area was measured to determine changes in morphology and migration studies were performed using Transwell permeable supports.
Functional characterization demonstrated sexually dimorphic patterns in our model. In phagocytosis assays, female microglia exhibited higher capacity to engulf E. coli spheres, but this advantage was lost after KIF21B silencing. With neuronal debris, KIF21B silencing reduced phagocytosis exclusively in female microglia. Similarly, KIF21B silencing reduced cellular area and migratory capacity specifically in females. Male microglia maintained these functions despite KIF21B reduction, suggesting sex-specific compensatory mechanisms. Cytoskeleton characterization of the interaction between microtubules and microfilament revealed that while KIF21B association with microtubules remained stable regardless of sex or inflammatory context, its interaction with F-actin showed dynamic regulation, with decreased interaction in female microglia after LPS treatment.
These findings identify KIF21B as a key regulator of microglial cytoskeleton dynamics, functioning in a context-dependent and sexually dimorphic manner. Female microglia show greater functional dependence on KIF21B, revealing complex regulatory mechanisms that may contribute to sex differences in neuroinflammatory responses. Our results provide new insights into microglial cytoskeleton regulation, underlying sex-specific mechanisms of phagocytosis.

Lack of Gal3 Ameliorates Neuroinflammation in Frontotemporal Dementia Model

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Progranulin (GRN) insufficiency leads to frontotemporal lobar degeneration (FTLD) with TDP-43 and neuronal ceroid lipofuscinosis. In this study, brains from WT, Gal3KO, PGRNKO, and PGRNKO/Gal3KO mice (18 months old) were analyzed using immunofluorescence and confocal microscopy. Results revealed that in PGRNKO mice, Gal3 was primarily located in the corpus callosum, thalamocortical tract, and thalamus. Additionally, a pronounced microgliosis was observed in those regions. In the thalamus of PGRNKO mice, microglia exhibited a phenotypic shift characterised by increased TREM2 and CLEC7A expression. Furthermore, GPNMB, a microglial marker, was upregulated in the sensorimotor thalamic region in the absence of GRN, and its expression further increased in the absence of Gal3, suggesting a potential anti-inflammatory effect. Cortical levels of pro-inflammatory cytokines like KC/GRO, IL-6 and IL-12p70 are increased in absence of GRN, which are restored in PGRNKO/Gal3KO mice.

LIMP2/SCARB2 regulates autophagy in oligodendrocytes: impact on cerebrovascular disease

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Autophagy is a key process to adapt to the metabolic shortage imposed by cerebrovascular disease in brain cells. In addition, lysosomal integral membrane protein 2 (LIMP2/SCARB2) regulates oligodendrogenesis and affects vasculogenesis in zebrafish. Therefore, the objective of this work is to study the bidirectional relationship between autophagy in oligodendrocytes and cerebrovascular disease. Specifically, we are assessing how genetic (Scarb2) and environmental (hypoxia) determinants of cerebrovascular disease affect oligodendrocyte autophagy and physiology. Our results indicate that SCARB2 mainly localized to lysosomes and late endosomes of cultured oligodendrocyte progenitor cells (OPCs), being also present in autophagosomes and early endosomes, suggesting widespread roles for SCARB2 in the regulation of lysosomal networks. Intriguingly, SCARB2 re-distributed after 3h hypoxia, when autophagy flux increased in OPCs. Although SCARB2 overall expression did not change, its presence in autophagosomes decreased, while tending to increase in other types of vesicles. Our preliminary siRNA knock-down experiments confirm that SCARB2 regulates autophagy in OPCs in control and hypoxic conditions. We are now also assessing the impact of SCARB2 and autophagy in the proliferation and differentiation of OPCs. Interestingly, our preliminary data on autophagy deficient ATG4B KO mice indicate increased numbers of OLIG2+ cells in the corpus callosum, suggesting a role for autophagy in the regulation of oligodendrocyte survival. We next plan to assess the consequences of autophagic dysfunction in oligodendrocytes in the vascular compartment. The understanding of the mechanistic connections between oligodendrocyte autophagy, their physiology, and the vasculature may eventually aid on identifying new therapeutic targets for the treatment of cerebrovascular diseases.

Lipid-transfer protein VPS13A modulate mitochondrial metabolism and function in neurons: novel insights for Chorea-Acanthocytosis

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VPS13A is an intermembrane lipid-transfer protein whose loss-of-function mutations, causing the protein depletion, lead to chorea-acanthocytosis (ChAc), an ultra-rare autosomal recessive neurodegenerative disorder. The main neuropathological characteristic of ChAc is the selective degeneration of the striatum linked to the progressive adult-onset chorea and dystonia. Yet, the underlying pathological mechanisms are poorly understood. Our group recently described that VPS13A colocalizes with endoplasmic reticulum and mitochondria organelle in neurons. Besides, mitochondrial fragmentation has been described in VPS13A knockout cell line models. Here, we aimed to investigate the impact of neuronal VPS13A in mitochondrial homeostasis and function. To identify VPS13A-interacting partners in the mouse brain, we performed protein immunoprecipitation followed by mass spectrometry analysis. We used a shRNA-based VPS13A knockdown (KD) model in primary neuronal cultures to assess the effects of VPS13A KD in mitochondrial morphology, dynamics, and function. Mitochondrial metabolism was evaluated using Seahorse Analytics. Potential dysfunction mechanisms and mitochondrial stress were analyzed measuring mitochondrial membrane potential and reactive oxygen species, respectively. Our mass spectrometry analysis revealed that VPS13A mainly interacts with proteins involved in mitochondrial metabolism. In neuronal cultures, VPS13A downregulation reduced mitochondrial size and decreased mitochondrial fusion and fission dynamics. Energy metabolism studies showed decreased aerobic and anaerobic metabolism, reduced ATP production, impaired metabolic potential and altered general metabolic profile in VPS13A KD neurons. Furthermore, VPS13A KD induced mitochondrial membrane hyperpolarization and reduced the mitochondrial membrane potential depolarization speed. Moreover, we observed a higher production of mitochondrial reactive oxygen species in VPS13A KD neurons, indicative of increased oxidative stress. Taken together, our findings provide evidence that reduced VPS13A levels in neurons alter mitochondria morphology, disrupt energy metabolism, impair mitochondrial function, and elevate mitochondrial stress. These results highlight the key role of cellular lipid distribution in maintaining mitochondrial homeostasis and function. They also contribute to unveil the pathophysiological mechanisms underlying ChAc.

Localization of ENGRAILED-1 in control and sporadic amyotrophic lateral sclerosis (sALS) human spinal cord

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ENGRAILED-1 (EN-1), a highly conserved homeoprotein transcription factor, has been extensively investigated for their main functions throughout embryonic development. EN-1 participates in central nervous system patterning through axon guidance and cell lineage differentiation, but its roles in adult life are less understood. In the mouse spinal cord, En-1 expression in V1 interneurons is secreted to the interstitial space and it is internalized and accumulated in α-motor neurons (Lebœuf et al., 2023). Its depletion in the ventral midbrain led to dopaminergic neuron loss and symptoms similar to those described in Parkinson's disease in mice. However, the localization of EN-1 in human spinal cord and its role in motor neuron diseases such as ALS remains unknown. Thus, we mapped the localization of EN-1 in the human spinal cord of ALS patients and controls by immunohistochemistry. The results obtained show that: 1) interneurons of the anterior horn show a localization pattern of EN-1 protein predominantly in the nucleus, and with a punctate pattern in the cytoplasm, whereas motor neurons show an accumulation of EN-1 exclusively in the cytoplasm. The rest of the spinal cord cells do not show immunohistochemical reactivity; 2) The study reveals that motor neurons in patients with sALS do not exhibit EN-1 protein accumulation in the cytoplasm, while anterior horn interneurons display a normal immunohistochemical pattern.
This demonstrates that the data observed in murine models are also present in the spinal cord of humans. The absence of EN-1 in motor neurons of sALS spinal cords suggests that the decreased internalization capacity of EN-1 could be involved in the pathogenesis of ALS, and points to the possibility of using EN-1 protein accumulation as a target for the development of new therapies.

Funding: MINECO/AEI/FEDER (PID2020-11817RB-100 and PID2023-150680OB-I00), GVA (PROMETEO-CIPROM/2021/018). Cátedra sobre la Esclerosis Lateral Amiotrófica: Gregoria Ramos Gil.

Longitudinal study of the adult hippocampal neurogenic niche homeostasis in a mouse model of Alzheimer's disease

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In the hippocampal dentate gyrus (DG), adult hippocampal neurogenesis (AHN) occurs within a specialized environment, the neurogenic niche, comprising an extensive vascular network and glial cells. This study aims to identify the cellular mechanisms underlying AHN failure in Alzheimer's disease (AD) by longitudinally studying the components of the DG neurogenic niche.

We used a mouse model of AD (mice that overexpress glycogen synthase kinase 3 beta (GSK-3β)), and conducted a longitudinal study at 4, 12, 18, and 30 months of age (mo). Our results reveal significant alterations in glial populations and vascularization. In AD mice, astrocyte and microglia densities increase in the granule cell layer (GCL) at 4 mo but decrease in the subgranular zone (SGZ) by 12 mo, accompanied by morphological changes observed through 3D reconstructions. Oligodendrocyte precursor cells (OPCs) decrease in the GCL at 4 mo, while mature oligodendrocytes remain stable in the GCL but decline in the SGZ from 12 mo onwards.
These glial changes correlate with increased DG vascularization, particularly in the SGZ at 12 and 18 mo and the GCL at 12 mo. GCL blood vessels exhibit increased thickness at 12 and 30 mo, with elevated CD31 immunoreactivity at 30 mo.
The putative interaction of newborn dentate granule cells with glial cells was also examined. Astrocytes show increased overlapping with 1-week-old neurons in the AD model, which decreases in 8-week-old neurons in both AD and wild-type conditions. Conversely, overlap with UEA1+ blood vessels increases in 8-week-old neurons compared to 1-week-old ones in AD mice.
Finally, a detailed analysis of the hippocampal expression of 96 cytokines revealed a variable imbalance between pro- and anti-inflammatory signals in the AD model.

These findings bring to light the disruption of the DG neurogenic niche homeostasis in this mouse model of AD, potentially contributing to AHN impairments.

Mapping Wasteosome Distribution: Uncovering Glymphatic Insufficiency in Neurodegeneration

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The glymphatic system is essential for removing metabolic waste products from the brain, and its impairment has been linked to various neurodegenerative disorders. Wasteosomes (also known as corpora amylacea) have emerged as potential indicators of chronic glymphatic insufficiency. This study investigates the distribution of wasteosomes across multiple brain regions to explore their association with glymphatic drainage and neurodegenerative disease risk.

We analysed brain tissue from 185 donors — including cases with neuropathologically confirmed Alzheimer's disease, amyotrophic lateral sclerosis with TDP-43 proteinopathy, frontotemporal lobar degeneration with TDP-43, tau or FUS proteinopathy, and non-diseased controls. Wasteosome accumulation was assessed in 28 brain regions comprised in five distinct brain areas, using region-specific scoring systems. Statistical evaluations were performed via variance/covariance analysis and decision tree methods.

Our findings reveal that wasteosomes consistently accumulate in seven key brain regions associated with glymphatic drainage but not in the primary targets of the distinct neurodegenerative pathologies. Notably, wasteosome load was significantly higher in these regions across all disease groups compared to controls, a difference that could not be attributed to the age factor.

The increased presence of wasteosomes in the glymphatic-associated regions supports the hypothesis that chronic glymphatic insufficiency plays a fundamental role in neurodegenerative processes. These results highlight the potential of wasteosome load as a valuable biomarker for assessing glymphatic function and neurodegenerative disease risk.

Mechanical Cues Control Tau Nuclear Localization in Neurons

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Brain tissue undergoes dynamic mechanical changes throughout development, aging, and in response to disease. In neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease, and multiple sclerosis, alterations in brain tissue mechanics are observed in vivo and correlate with key histopathological features, including neuronal loss, disrupted connectivity, and inflammation. However, whether these mechanical changes are a consequence of disease progression or actively contribute to neurodegeneration remains an open question.
To investigate neuronal mechanosensing, we developed a differentiation protocol for SH-SY5Y cells that combines biochemical induction with NGN2 overexpression, promoting rapid and enhanced neuronal maturation. Differentiated cells were cultured on polyacrylamide gels with varying rigidities functionalized with laminin and fibronectin. Neuron-like cells grown on softer substrates exhibited reduced spreading, shorter neurite length, and decreased polarity in the distribution of stable microtubules, reflected by a lower neurite/soma ratio. Strikingly, compliant environments induced nuclear accumulation of Tau phosphorylated at residues 202-205, a hallmark of AD pathology. Pharmacological inhibition revealed that this nuclear translocation of phosphorylated Tau is regulated by microtubule stability rather than actin dynamics.
To validate these findings in a disease-relevant model, we used human induced pluripotent stem cell (hiPSC)-derived neurons from an AD patient with the APOE4/E4 genotype and its isogenic APOE3/E3 control. APOE4/E4 neurons exhibited enhanced endosomal accumulation, increased accumulation of perisomatic and nuclear Tau phosphorylation, and impaired polarity of microtubular stability, all of which were exacerbated on softer substrates.
These results suggest that neuronal mechanotransduction plays a crucial role in AD-related Tau mislocalization, linking brain tissue mechanics to cytoskeletal dysfunction in neurodegeneration. Consequently, targeting mechanosensitive pathways may offer novel therapeutic strategies for AD.

Meta-Analysis of Microglial Single-Cell RNA Sequencing in Mouse Models and Human Alzheimer's Disease

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by progressive cognitive decline, amyloid-beta (Aβ) plaque deposition, neurofibrillary tangles, and chronic neuroinflammation. Microglia, the resident immune cells of the central nervous system, play key roles in brain homeostasis by surveying their environment, clearing extracellular protein aggregates, and modulating neuroinflammatory responses. Emerging evidence suggests that mutations in microglia-associated genes significantly impact AD development by altering key functions such as cytokine secretion and phagocytic activity. These dysfunctions contribute to increased neuroinflammation and impaired clearance of Aβ peptides, exacerbating AD pathology. Single-cell and single-nucleus RNA sequencing (sc/snRNA-seq) have provided unprecedented insights into microglial heterogeneity in both AD mouse models and human patients. However, inconsistencies in nomenclature, divergent microglial populations, and discrepancies between species have hindered a unified understanding of microglial states in AD pathogenesis. Here, we present a meta-analysis of publicly available sc/snRNA-seq datasets to identify conserved and divergent microglial transcriptional signatures across AD mouse models and human AD brains. By integrating multi-study datasets, we aim to refine microglial classification and delineate shared and species-specific microglial populations.

Metabolic modulation through pharmacological HIF1α stabilization reduces Alzheimer's Disease pathology

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Currently, most emerging therapies against Alzheimer's Disease (AD) aim to block or reduce beta-amyloid and phospho-tau pathology, while only some of them target neuroinflammation. However, none has achieved significant overall success. Given that mitochondrial activation of microglia and astrocytes correlates with neuroinflammation and AD progression, we propose a novel treatment based on metabolic modulation via HIF1α stabilization. The presence of HIF1α in glial cells avoids mitochondrial overactivity, returning them to a surveilling role.

In vitro, we stimulated primary microglia using extracellular tau vesicles and oligomeric beta-amyloid. Both treatments triggered a strong inflammatory response. Nevertheless, a 12-hour-preincubation of our microglia with a drug which promotes HIF1α stabilization enhanced the phagocytosis and processing of beta-amyloid, modulated the neuroinflammatory response, and downregulated the AKT-mTOR signaling pathway. Additionally, microarrays analysis and respiration assays demonstrated a significant shift from oxidative phosphorylation to glycolysis in both pathological and homeostatic conditions. Otherwise, a 12-hour incubation of SH-SY5Y-Tau neuronal cultures, which overproduce tau, reduced tau aggregation, and phosphorylation, suggesting an increased capacity of clearance in these treated neurons.

In vivo, 21 days of intraperitoneal administration of the same compound in 18-month-old APP751SL mice improved behavioral parameters, as assessed by the nesting and open field tests. These changes correlated with a reduction in hyperphosphorylated tau and total beta-amyloid, alongside improvements in synaptic VGAT and VGLUT levels, confirmed by western blot. The treatment also reduced the activation of the mTOR pathway, indicating a metabolic rewiring. Furthermore, microglial activation markers (Clec7a, Cd45) and pro-inflammatory cytokine levels (Il-1β) measured by RT-qPCR were reduced, while astrocytic reactivity (Gfap, Vim) decreased as well.

Our findings suggest that mitochondria influence glial cell activation, making HIF1α stabilization a promising strategy for AD. However, further studies are required to validate this hypothesis.

Microglia indirectly promote regenerative neurogenesis by modifying signalling from regeneration-specific neurons after spinal cord injury in zebrafish

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After central nervous system injury, mammals fail to regenerate neurons. In contrast, zebrafish regenerate neurons, such as motor neurons, from ependymoradial glial cells (ERGs) after spinal injury. Microglia potentially regulate the neurogenic capacity of ERGs through several signals. At the same time, a population of neurons, which is induced by an injury, inhibits neurogenesis by secreting the neuropeptide pyyb (manuscript in preparation). Analysing scRNAseq data indicates potential cross-talk of microglia and lesion-induced neurons via kit ligand (kitlg) /kit receptor signalling. In situ, kitlga is highly enriched in microglia and kitb in lesion-induced neurons. Somatic mutation of ligand and receptor reduce regenerative neurogenesis from ERGs. Mutation of kitb also increases expression of pyyb. Hence, microglia likely promotes regenerative neurogenesis by attenuating inhibitory pyyb signalling from lesion-induced neurons through kit signalling. This indicates a unique cellular and molecular signalling environment in regenerative neurogenesis.

Funded by Alexander von Humboldt-Stiftung, TU Dresden core fundings and Tenovus Scotland Medical Research Charity.

MICROGLIAL AHR DRIVES ALZHEIMER'S DISEASE PROGRESSION

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by amyloid-β (Aβ) plaques, neurofibrillary tangles, and neuroinflammation. Microglia play a key role in AD by accumulating around Aβ plaques, contributing to both Aβ clearance and neurotoxicity. Emerging evidence suggests gut microbiota metabolites (GMMs) influence microglia but the way microglia respond to GMMs remains an unanswered question. A possibility is that this regulation occurs through the Aryl Hydrocarbon Receptor (AhR), a ligand activated transcription factor, which may serve as a molecular link between GMMs and microglial responses. Constitutive and microglia-specific AhR-KO mice were generated and crossed with APPNL-Fmice to assess AhR's role in AD progression. AhR was activated using indoxyl 3-sulfate (I3S). AD hallmarks were evaluated by immunofluorescence, WB, ELISA, flow cytometry. Microglial function was assessed through phagocytosis assays. scRNA-seq was performed to characterize transcriptional changes in microglia. AhR ligand activity was measured in APPNL-Fmice and patient samples using a luciferase-based AhR agonist assay. GM composition was analyzed via 16S-rRNA sequencing. AhR metabolites were identified using untargeted metabolomics.
Our data demonstrate that AhR expression is upregulated in the cortex of APPNL-F AD mice, primarily in microglia closely associated with amyloid plaques. Using constitutive and microglia-specific AhR-/- models, as well as activation with the I3S, we show that AhR deletion leads to a significant reduction in amyloid pathology, dystrophic neurites, and cortical Aβ42 levels. Conversely, AhR activation further supports its detrimental role in AD progression. Moreover, AhR modulates glial function, driving a reactive phenotype, impairing microglial phagocytosis. scRNA-seq confirms that AhR acts as a transcriptional driver of glial responses in AD. Strikingly, luciferase assays reveal increased AhR activity in samples from AD mice and patients. Additionally, 16S-rRNA sequencing and untargeted metabolomics identify GM-derived tryptophan metabolites as key AhR ligands. In conclusion, AhR plays a pivotal role in the development and progression of AD.

Mislocalized cytoskeletal protein biogenesis within TDP-43 aggregates derails sarcomere organization in human myocytes induced from fibroblasts

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A key challenge in the study of amyotrophic lateral sclerosis (ALS) is that the affected cells, mainly human motor neurons and muscle cells, are difficult to isolate and culture. To address this limitation, we evaluated cultured dermal fibroblasts as a potential cellular model for ALS investigations, given their accessibility via minimally invasive biopsies and their capacity to be reprogrammed into ALS-relevant cell types, including neurons and muscle cells. Morphological and biochemical analyses of patient-derived fibroblasts revealed prominent cytoplasmic mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43), along with substantial disruption of F-actin and 4A-alpha-tubulin fibers, indicating compromised cytoskeletal integrity, which are pathological features of the disease. Intriguingly, cytoskeletal fibers partially colocalized with cytoplasmic TDP-43, including within its aggregates, suggesting that TDP-43 may participate in their synthesis or assembly in control cells and contribute to their mislocalization and disorganization in the ALS context. To further investigate these observations, we reprogrammed fibroblasts into induced myocytes (iMCs) via retroviral expression of the muscle lineage determinant MyoD. Consistent with previous findings, control iMCs exhibited TDP-43 not only in the nucleus but also in association with myosin within sarcomeric structures, implying a role in muscle skeletal protein biogenesis, particularly within the sarcomere. Moreover, in patient-derived iMCs, myosin colocalized with TDP-43 aggregates while sarcomeres were disrupted, supporting the notion that these aggregates misdirect myosin synthesis and disrupt sarcomere organization. Finally, we investigated whether preventing TDP-43 aggregation could reduce myosin aggregation and restore sarcomere structure. To this aim, we used protein kinase inhibitors, as our previous work demonstrated their ability to restore TDP-43 nuclear localization and promote aggregate clearance. Treatment with these inhibitors rescued TDP-43 distribution and alleviated cytoskeletal defects. Overall, our findings reveal a novel mechanism that may be central to ALS, deepening our understanding of disease pathogenesis and identifying new avenues for therapeutic intervention.

Modelling Parkinson's Disease (PD) and Alzheimer's Disease (AD) using assembloids derived from human pluripotent stem cells

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Over the last decade, cerebral organoids derived from human induced pluripotent stem cells have become the most promising in vitro tool to investigate human neurodegenerative diseases. Their three-dimensional architecture provides spatial information that is missing in other stem cell-derived cultures, making it possible to study cellular diversity and intercellular interactions in vitro. Additionally, organoids from distinct brain regions, can be fused to create assembloids, in which cells can migrate and establish connections between different regions, modelling the neural circuits of the human brain. In this project, we have developed an in vitro model to study Parkinson's disease (PD) and Alzheimer's diseases (AD) by differentiating and fusing cortical, striatal and ventral midbrain organoids. Next, we have exposed the assembloids to alpha-synuclein (alpha-syn) preformed fibrils (PFF) and Tau aggregates, in order to analyze their neurotoxic effect in the nigrostriatal pathway (in PD) and cortex (in AD). Our preliminary results have revealed that the developed organoids express region-specific markers corresponding to the cortex, striatum and midbrain, pointing out to a correct differentiation of the model. Additionally, assembloids exhibit one of the PD hallmarks: a reduction of tyrosine hydroxylase (TH)-expressing dopaminergic neurons after their exposure to alpha-syn PFFs. Future perspectives include assessing the effects of Tau exposure and conducting a more comprehensive characterization of the molecular response to both alpha-syn and Tau aggregates, to determine whether this novel cellular model can replicate other neurotoxic processes described in PD and AD, such as the damage to neuronal circuits.

Modulating Microglia to Mitigate Neurodegeneration in Alzheimer's Disease

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Microglia are the main primary immune cells of the brain and play important homeostatic functions that seem to be lost in neurodegenerative diseases, including Alzheimer's Disease (AD). Persistent microglial activation has been widely described in different AD transgenic mice, creating a chronic neuroinflammatory environment in the brain that could contribute to the progression of the disease. Moreover, we have described a microglial degenerative process in the hippocampus of AD patients, mainly mediated by pathological Tau species. All those reasons have raised interest in the development of microglial depletion approaches and their use in our understanding of the disease. PLX3397 is an oral tyrosine kinase inhibitor widely used for microglial depletion. It is in phase 1-3 clinical trials for treating different cancers and produces CSF1R inhibition, an essential receptor for the viability and proliferation of these cells. In accordance with other reports, we have observed that a rapid and complete repopulation follows microglial depletion. Since the phenotype of these emerging cells and its effect on the pathology is still unknown, we decided to perform a two-week PLX3397 treatment in both, hTau P301S and APP aged mice models of AD. Although we didn't see any differences in the amyloid or tau load in the depleted and repopulated mice, we did observe a reduction in the main inflammatory cytokines. Even though the emerging microglia exhibit a less activated phenotype, they appear to fulfill the functions of resident microglia. Both, hyperactivated and dysfunctional microglia seem to disrupt homeostasis in the brain with the progression of the pathology, as a result, microglial renewal may be a promising therapeutic strategy for AD.

Modulation of neuroinflammatory response by Enriched Environment in alpha-synuclein induced Parkinson's disease model

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Neuroinflammation is a critical factor in the onset and progression of Parkinson's disease (PD), a common neurodegenerative disorder affecting 1–5% of individuals over 60, with a higher prevalence in men. Beyond the hallmark motor symptoms—tremors, rigidity, slow movements, and gait disturbances—PD is characterized by the accumulation of misfolded α-synuclein (α-syn) in Lewy bodies and neurites. While α-syn serves essential physiological functions, its aggregation triggers neuroinflammatory responses that contribute significantly to neuronal damage.
Chronic immune activation in the brain, driven by microglial reactivity and elevated pro-inflammatory cytokines, accelerates neurodegeneration. Understanding how to regulate these processes is key to developing new therapeutic strategies. Recent research highlights the potential of enriched environments (EE) to counteract inflammatory cascades and promote neuroprotection in neurodegenerative diseases.
This study investigated the impact of EE on brain immune responses in a rat model of PD with α-syn overexpression. Using adeno-associated viral (AAV9) vectors, α-syn was bilaterally injected into the substantia nigra of two-month-old male Sprague Dawley rats. Post-injection, rats were divided into two groups: standard conditions and EE. After two months, markers of glial activation (IBA-1) and pro-inflammatory mediators like IL-6 were assessed using optical densitometry and western blot.
The findings revealed that EE significantly reduced the inflammatory processes induced by α-syn overexpression, restoring IBA-1 and IL-6 levels to physiological ranges in the striatum and substantia nigra. These results underscore the crucial role of neuroinflammation in PD progression and demonstrate the potential of enriched environments to modulate this process, offering promising insights for future non-pharmacological therapeutic approaches.
This work has been supported by a grant PID2021-126434OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and ERDF/EU. It has also been funded by the Basque Government (IT1706-22, PUE21- 03). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) "non-motor Comorbidities in Parkinson's Disease (CoMorPD)".

Modulation of the Foreign Body Reaction against Intraneural Electrodes with Inhibitors of Inflammasome

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The implantation of an electrode or any medical device triggers a foreign body reaction (FBR) as part of the body's defense mechanism. This process involves the activation of macrophages and giant cells, followed by the differentiation of fibroblasts into myofibroblasts to heal the wound and encapsulate the implanted material. However, this FBR is detrimental to neural electrodes, as it reduces their ability for stimulating nerve fibers and for recording nerve impulses over time. The FBR can be modulated through systemic or local administration of drugs aimed at mitigating or inhibiting inflammation.
In this context, we have developed an in vitro model of reactive macrophages activated by lipopolysaccharide (LPS) exposure to study the inflammatory phenotype in the presence of inflammasome inhibitors, such as MCC950 and its novel synthetic analogue, TDV19. First the compounds were tested added to the culture medium, where they reduced inflammasome activation, leading to a decrease in the expression of cytokines IL-1ß, IL-18, and a reduction in caspase-1 activation. Furthermore, immunohistochemical analysis showed that both compounds promoted the expression of anti-inflammatory markers (such as CD206) and reduced the expression of pro-inflammatory markers (e.g., iNOS). Subsequently, in vivo studies were conducted in which polyimide LIFE devices were implanted into the sciatic nerve of rats, and the same drugs were administered to the animals. The results showed a significant reduction in macrophage recruitment in the implanted nerve, and a reduction of the size of the capsule surrounding the implant at 2- and 8-weeks post implantation. Both in vitro and in vivo results, demonstrated a significant reduction in inflammatory activation and infiltration, suggesting that these drugs are potential candidates for reducing fibrosis on active electrodes implanted in the nervous system.

MOLECULAR BASIS FOR THE ALTERATION OF FUNCTIONAL COUPLING OF NMDA RECEPTORS AND BK CHANNEL IN THE FRAGILE X SYNDROME

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FMRP, the protein involved in Fragile X syndrome, is mainly known as a ribonucleoprotein, and has been suggested to play roles in nucleocytoplasmic shuttling of mRNA, dendritic mRNA localization, and synaptic protein synthesis. However, FMRP is able to directly interact with ion channels. Our group has demonstrated that the function of BK channels is regulated by FMRP (Kshatri et al., 2020). On the other hand, functional coupling between NMDA receptors and BK channels has been shown to have an important role in synaptic function (Gomez et al., 2021; Martinez-Lazaro et al., 2025). Interestingly, work from our group demonstrates that NMDAR-BK coupling is altered in several brain regions of FXS mouse model (Fmr1-KO). In this work, we investigate the interaction between FMRP, BK and NMDA receptors in a cell heterologous system, where we characterize the physical and functional interactions between FMRP, BK and NMDAR. To this end, we use electrophysiology (whole-cell recordings), live cell imaging (calcium imaging with the genetically encoded calcium indicator Lyn-R-GECO1), proximity ligation assay (PLA) to detect protein interactions/distances and co-immunoprecipitation (co-IP). Our results show that the lack of FMRP associated to the FXS may result in malfunction of the NMDAR-BK macrocomplexes, offering possible mechanisms underlying altered synaptic function in this pathology.

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Motor cortex disinhibition linked to olfactory dysfunction in Parkinson's Disease

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Motor cortex disinhibition, characterized by impaired short-interval intracortical inhibition (SICI) as measured by transcranial magnetic stimulation (TMS), is a well-recognized feature of Parkinson's disease. However, significant variability among patients remains unexplained, raising questions about its underlying mechanisms and clinical relevance. Given the established biological links between olfaction and motor function, this study aimed to explore the relationship between motor cortex disinhibition and olfactory dysfunction in Parkinson's disease. We assessed these features in a cohort of early-to-mid-stage patients (n=45) and age- and gender-matched healthy controls (n=35) using SICI to evaluate cortical inhibition and the Sniffin' Stick Test 12 items (SST-12) to measure olfactory function. As expected, patients exhibited reduced cortical inhibition and impaired olfactory function, with moderate-to-extreme Bayesian evidence supporting these findings. Interestingly, neither feature correlated with clinical motor severity. However, a strong-to-extreme correlation was observed between cortical disinhibition and olfactory dysfunction across all subjects, within the patient group, the control group, and the subgroup of levodopa-naïve patients (n=20). Age was tested and ruled out as a potential confounding factor. Causal inference analysis suggested that Parkinson's disease mediated this association in a manner consistent with an internal pathogenic mechanism rather than an external influence. These findings support the notion that motor cortex disinhibition and olfactory dysfunction may be linked through a common early pathogenic process in Parkinson's disease, providing insight into disease heterogeneity and potential early biomarkers.

Multiomic analyses identify ICAM1 as a Potential Mediator of Astrocyte-Driven Neurotoxicity in Alzheimer's Disease

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Astrocytes are central players in the pathogenesis of Alzheimer's disease (AD), undergoing profound changes in gene expression, morphology, and function. These changes can significantly alter the astrocyte-neuron microenvironment, yet remain poorly understood. Using human pluripotent stem cell-derived astrocytes (hAstrocytes), we established an in vitro approach to explore astrocyte responses to AD-relevant challenges and their impact on neurons
Our data reveal that conditioned medium from hAstrocytes (ACM) exposed to AD associated factors induces significant neuronal death, in contrast to the lack of toxicity observed when neurons are directly exposed to the same challenges. This highlights a secreted, hAstrocyte-dependent mechanism of neurotoxicity. Multiomic analyses of hAstrocytes treated with these AD-relevant factors revealed significant dysregulation of pathways involved in inflammatory processes, extracellular matrix remodeling, and neuronal maintenance. Among the secreted factors, ICAM1 was identified as a key candidate mediating hAstrocyte-induced neurotoxicity.
These findings provide new insights into the role of hAstrocytes shaping a neurotoxic environment in AD and identify ICAM1 as a potential driver of astrocyte-neuron interactions in neurodegeneration. In addition, this model serves as a valuable platform for further investigation into hAstrocyte-mediated mechanisms of AD pathology.

Multi-omic approach highlight specific molecular signature in Parkinson's disease patients-derived dopaminergic neurons carrying new genetic rare variants

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Parkinson's disease (PD) pathophysiology is still an aim of study; PD is characterized by an interplay of several altered cellular mechanisms. In the last years, new genes emerged associated to PD insurgence. Recently, we demonstrated the existence of a polygenic model of inheritance associated to both familial and sporadic PD forms. In this study we generated 10 induced Pluripotent Stem Cells (iPSC) from 6 PD patients carrying rare mutations in new genes such as AIMP2, HMOX2, SLC6A3, KIF21B, LRRK2, RHOT2, TMEM175, TOMM22, TVP23A, ZSCAN21 and 4 matched healthy subjects. We used these iPSCs-derived mesodiencephalic dopaminergic neurons (mdDA) to disclose deregulated pathways and monitor their electrophysiological properties. Patch-clamp experiments disclosed a defect in current recording and cell capacitance in patient-derived neurons when compared to healthy subjects. Particularly, patients carrying heterozygous mutations in SLC6A3 and in HMOX2 genes displayed a more pronounced electrophysiological immature profile compared to those of other patients and controls. Furthermore, we also integrated data from untargeted proteomic and lipidomic approach with transcriptomic analysis of mdDA neurons and we revealing a common signature of altered transcript, proteins and lipids involved in the most relevant step of cellular energy production and related organelles like mitochondria and peroxisomes, affecting the most of PD patients-derived mdDA neurons. These findings were also supported by expression studies performed with droplet digital PCR. In conclusion, we highlight that multilayer integrative analysis could corroborate new potential cellular mechanisms and target in support of personalized medicine application for PD diagnosis and prognosis.

Nerve regeneration within a chitosan conduit after immediate and delayed nerve repair

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Peripheral nerve injuries represent a significant clinical challenge due to their limited regenerative capacity. Surgical repair strategies include immediate nerve repair, performed shortly after injury, and delayed nerve repair, conducted after a prolonged period when primary intervention is not feasible. Understanding the molecular and genetic mechanisms underlying both strategies is essential for optimizing functional recovery. This study compares the transcriptional landscape of delayed and immediate nerve repair over time to elucidate key pathways involved in nerve regeneration.

A rat model of sciatic nerve injury was used, where an 8-mm nerve gap was repaired using a chitosan conduit either immediately or after a 3-month delay. Regenerated nerves were collected at 7-, 14-, and 21-days post-repair. RNA sequencing was performed to analyze transcriptomic changes across time points, and differentially expressed genes were identified and subjected to Gene Ontology enrichment analysis to identify functional pathways and regulatory networks involved in nerve regeneration.

Distinct transcriptional profiles were observed across time points after immediate and delayed repair when comparing regenerating nerves with a healthy nerve. At 7 days, both exhibited a robust inflammatory response with upregulation of genes related to cytokine signaling and myeloid cell activation. By 14 days, many pathways related to response to lipoprotein particles and phagocytosis were upregulated, with delayed repair showing downregulation of myelination-associated genes. At 21 days, immune response activation, phagocytosis and lipoprotein particle activated pathways remained upregulated in both conditions.

These findings suggest that immediate repair induces a faster regenerative response, whereas delayed repair follows a slower trajectory but engages similar molecular pathways. Despite initial differences, both strategies eventually converge on key regenerative mechanisms. These insights emphasize the benefits of early intervention while highlighting the potential for delayed repair to achieve functional recovery. Future research will focus on enhancing delayed repair outcomes through targeted molecular approaches.

Neurodegenerative diseases: what can be learned from toothed whales?

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Research has shown evidence of neurodegeneration across various animal species, particularly in marine mammals. The order Cetacea comprises two suborders: Mysticeti (baleen whales) and Odontoceti (toothed whales). Toothed whales (TW), being apex predators, function as environmental indicators and can provide valuable insights into human diseases. Current research indicates that certain marine mammals, specifically TW, display neuropathological characteristics similar to Alzheimer's disease (AD), potentially offering new perspectives on neurodegenerative conditions. Hypoxia, resulting from prolonged deep dives, is a significant risk factor, particularly for deep-diving TW species. While TW brain exhibits some protective adaptations against mild hypoxia, prolonged or severe hypoxia can lead to neuronal damage and accelerate the development of NDDs. The concept of “neuronal selective vulnerability” is explored, focusing on the susceptibility of specific brain regions, such as the Purkinje cells of the cerebellum and hippocampal neurons, to hypoxia and toxic insults. The presence of neuromelanin (NM) in TW is discussed, highlighting its potential role in neuronal vulnerability and the development of Lewy body pathology. Additionally, NM displays distinct features in humans and primates that are absent in other species. Initial observations of NM in the Delphinidae family were followed by transmission electron microscopy studies revealing melanin granules connected to lipid droplets and membranes in two TW species, showing remarkable similarity to human NM. Additional research on TW examined α-synuclein, ubiquitin, and laforin, revealing α-synuclein/ubiquitin immunopositive spherical structures within the mesencephalon's neuropil. TW exhibit several features consistent with human NDDs and more research is needed to fully understand the underlying mechanisms and the extent to which these pathologies are analogous to human diseases. While transgenic animals offer clear benefits for research, studying natural, non-transgenic models might provide relevant insights into the pathophysiology of neurodegenerative disorders.

Neuronal deficiency of the lipid transfer protein VPS13A disrupts diacylglycerol / Protein kinase C signaling in a model of chorea-acanthocytosis

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Chorea acanthocytosis (ChAc) is an ultrarare inherited neurodegenerative disease caused by a VPS13A gene mutation, resulting in the loss of the VPS13A protein. This leads to a selective and progressive neurodegeneration of the striatum and, consequently, to a dysfunction of the corticostriatal pathway. VPS13A functions as a bulk lipid transporter between different organelles, suggesting that neuronal lipid dyshomeostasis may be a key pathological process in ChAc. However, the precise role of VPS13A in lipid function in neurons remains unknown.
To elucidate this, we established a shRNA-based knockdown (KD) mouse model and investigated the effect of VPS13A KD on mouse brain lipid concentration in an open-hypothesis lipidomic analysis. We identified 173 different glycerophospholipid and sphingophospholipid species, along with some of their precursors and derivatives, in the brain tissue of KD and Control (Ctrl) mice. Analysis of differential concentration revealed significantly elevated levels of 14 different diacylglycerol (DAG) species in KD brain tissue. Then, we explored the metabolic pathways and downstream molecular pathways that are related to the altered diacylglycerol levels and found that VPS13A knockdown induces a decrease in protein kinase C (PKC)β concentration but an increase in PKCα/β phosphorylation in cultured neurons. Specific pharmacological inhibition of PKCβ induced aberrant neuronal morphology in VPS13A KD neurons but not in controls. These results provide evidence of the important role lipid cellular distribution mechanisms play in regulating synaptic plasticity processes. Our results also uncover the deregulation of diacylglycerol / PKC signaling as a new pathophysiological mechanism driving neuronal dysfunction and degeneration in ChAc.

Neuronal Diversity and Transcriptomic Alterations in the Human Dorsal Striatum in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder marked by motor dysfunction due to dopaminergic neuron degeneration in the substantia nigra and Lewy body formation. Dorsal striatal circuits, critical for movement control, are disrupted in PD.The irreversible loss of the dopamine neurons results in a reduction of the control exerted by the striatum, which is considered the main input structure for the basal ganglia system, as it integrates cortical inputs and has a major role on the fidelity of movement.
Using single-nucleus RNA sequencing (snRNA-seq) of post-mortem human caudate nucleus and putamen, we mapped striatal neuronal diversity, identifying eight major interneuron classes subdivided into fourteen cell types and six MSN cell types. Focusing on PD-associated alterations, we discovered three dysregulated interneuron subtypes (PVALB, PTHLH/MOXD1, and TAC3) and three MSN subtypes (D1-PCDH11X, D1-PTPRQ, and D1D2) with more than 300 differential expressed genes. PVALB interneurons exhibited a unique downregulation pattern especially affecting pathways related with protein metabolism and mitochondrial function, while TAC3 and PTHLH/MOXD1 shared a common dysregulation profile. As for MSNs, D1D2 MSNs showed enhanced cell-cell communication across all neuronal populations, D1-PTPRQ displayed altered synaptic function, and D1-PCDH11X showed upregulation in pathways linked to protein synthesis and mitochondrial metabolism. Furthermore, module analysis identified a PD-associated gene expression module enriched for antisense transcripts and long non-coding RNAs (lncRNAs) across all neuronal types.
Spatial transcriptomic analysis using the Xenium in situ platform validated our described neuronal subtype composition, as well as disease-related traits.It also provided spatial context for these populations within the striatum. This integrative approach strengthens the robustness of our snRNA-seq findings.
Our study provides a high-resolution atlas of striatal neuronal subpopulations in PD, linking cellular diversity to molecular pathology, highlighting the vulnerability of specific MSN and interneuron subtypes. This work advances the understanding of striatal composition overall and its contributions to PD.

Neuroprotective and myoprotective effects of the alkaloid boldine in adult and aged mice

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Aging is characterized by a decline in regenerative and protective capabilities, leading to both cognitive and motor impairments. Boldine, an alkaloid derived from the Peumus boldus tree, exhibits significant antioxidant and anti-inflammatory properties. It also inhibits hemichannels that remain opened under pathological conditions, thereby reducing oxidative stress and excitotoxicity without blocking gap junction-mediated cell communication. In addition, boldine has demonstrated neuroprotective effects in Alzheimer's disease, improved learning and memory, and beneficial impacts on skeletal muscle. These findings prompted us to investigate the effects of boldine on glial activation, neurogenesis, and cognitive and motor functions in aged mice.

Adult and aged C57/BL6 mice (3 and 23 months old) received a daily oral dose of boldine (50 mg/kg) or vehicle for one month. Neuroinflammation was evaluated using immunohistochemistry and western blotting for GFAP and Iba1. Muscle function was assessed through grip strength test and histological analysis of skeletal muscle, including the detection of tubular aggregates using a modified Gomori Trichrome technique.

Boldine treatment in adult mice led to a reduction in glial reactivity in the hippocampus and cerebral cortex, indicating an anti-inflammatory effect in the brain. This was confirmed by immunofluorescence, which showed a decreased density of reactive astrocytes and microglia in these regions compared to the control group.

Regarding muscle function, aged mice treated with boldine exhibited a significant increase in fall latency, relative to body weight, compared to vehicle-treated mice. Histological analysis of skeletal muscle revealed a significant decrease in the presence of tubular aggregates, which are indicative of age-related muscle alterations such as cellular proteostasis dysfunction, in boldine-treated mice compared to controls.

These results demonstrate the neuroprotective and muscle-preserving effects of boldine in both adult and aged mice, highlighting its potential as a therapeutic strategy to mitigate age-related decline in the nervous and muscular systems.

Neuroprotective effect of pterostilbene in a murine model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is the most common form of human epilepsy, affecting over 70 million people worldwide. TLE is characterized by recurrent epileptic seizures, primarily impacting the limbic system, including the hippocampus. Current therapy for this disease is based on the use of antiepileptic drugs; however, 30-40% of patients are drug-resistant to treatments. Understanding the pathways of cell damage following seizures is crucial for developing new drugs that are not pharmacoresistant and possess high absorption and bioavailability to reverse neuronal damage swiftly. Natural compounds, like stilbenes, that can cross the blood-brain barrier hold significant promise. Specifically, Pterostilbene (PTE) has been shown to reduce cognitive decline, enhance memory consolidation, and improve neurodegenerative conditions. These results have prompted us to investigate whether PTE can reverse neuronal damage after an epileptic seizure.
To this end, two approaches have been taken: 1-Compare the bioavailability of PTE and its cocrystal Pterovita (which has increased bioavailability) in blood and brain. 2-Study the effects of PTE when administered in a murine model that replicates temporal lobe epilepsy in humans.
Pharmacokinetic analyses of native PTE and Pterovita were performed in 2-month-old mice using high-performance liquid chromatography (HPLC). To assess neuroprotection, kainic acid (KA), a well-established TLE model, was administered intraperitoneally. PTE was given at different intervals before and after KA exposure.
HPLC results showed that Pterovita is better absorbed than PTE, reaching peak concentration (Cmax) faster, though no significant differences in overall Cmax levels were observed. Post-seizure PTE treatment prevented neurodegeneration and microgliosis, while pre-seizure administration (30 min before KA) reduced activation of rapid signalling pathways like NF-kB and iNOS.
These findings underscore the potential of PTE as a therapeutic agent to counteract neuronal damage in TLE, highlighting the promise of natural compounds in epilepsy treatment.

Neuroprotective Effects of D-chiro-inositol in Experimental in vitro Models of Alzheimer's Disease Improves Amyloid pathology, Structural/Functional Plasticity of Synapses and Impairment in the PI3K-Akt-mTOR Signaling Pathway

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Alzheimer's disease (AD), is a neurodegenerative disorder associated with deposition of amyloid beta (Aβ) peptides. Soluble oligomers, especially Aβ42, have a high neurotoxicity responsible for synaptic degradation and memory impairment. Clinical studies have shown several enzymes that contribute to Aβ degradation, such as insulin degrading enzyme (IDE), which degrades not only insulin, but also Aβs. It represents one of the promising links between Type 2 Diabetes Mellitus and AD.
In this study, we examined the action of D-Chiro-inositol 10 µM (DCI), an insulinmimetic inositol used against insulin resistance, in primary neuron culture from AppNL-G-F mice. Also human neuroblastome cells (SH-SY5Y) overexpressing amyloid precursor protein with the Swedish mutation (hAPPswe) were characterised. An ELISA kit was used in the detection of secreted Aβ42 and Aβ40 levels in the cell culture supernatants from AppNL-G-F and WT mice after 4 days of DCI incubation. Additionally, we studied the neuroprotective effect of DCI treatment in neurite outgrowth and synaptic functions through MAP2 immunofluorescence. Moreover, we determined the modulation of the impairment in the PI3K-Akt-mTOR signaling pathway in the SH-SY5Y cell line, via Western blot analysis.
Our results showed a significant reduction in Aβ42 and Aβ42/ Aβ40 ratio in the AppNL-G-F-DCI neuronal media group compared to the AppNL-G-F-vehicle group. We also observed a genotypic effect in the fluorescence intensity of neuronal soma as in the length of neuronal dendrites in AppNL-G-F vs WT mouse neurons. After DCI treatment, these alterations were reversed in AppNL-G-F-DCI neurons. Two-way ANOVA showed overexpression in proteins associated with the insulin signaling pathway (mTOR, IDE, IR-β, PI3K, AKT and GSK-3β), in the SH-SY5Y hAPPswe-vehicle group compared to the WT-vehicle group. DCI treatment modulated insulin dysregulation via PI3K-Akt-mTOR pathway. In conclusion, our study suggests that DCI exerts neuroprotective properties though a neuronal in-vitro model, and improves insulin resistance via SH-SY5Y cells.

Neurotropism and neuropathological alterations associated to different SARS-CoV-2 variants of concerns in susceptible transgenic mice

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Coronavirus disease 2019 (COVID-19) is caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although COVID-19 is primarily a respiratory disease, neuropsychiatric symptoms are common in patients, even after recovering from a breakthrough infection, suggesting the possibility of chronic brain impairment associated with long COVID-19 syndrome. Direct SARS-CoV-2 brain infection has been demonstrated in different experimental animal models and natural hosts of the virus, such as hamsters, ferrets, and non-human primates. Initial neuropathological studies on deceased COVID-19 patients, during the prevalence of the ancestral Wuhan strain, showed a significant SARS-CoV-2 neurotropism. However, subsequent investigations conducted during the predominance of variants of concern (VoCs) failed to detect cerebral infection in COVID-19 brain autopsies, suggesting differences in neuroinvasiveness and neurotropism among VoCs. Since we previously reported brain infection and damage caused by the ancestral Wuhan strain in susceptible K18-hACE2 transgenic mice, in this study, we performed a comparative analysis of the neurotropism of multiple SARS-CoV-2 VoCs and subvariants of concern, including BA.1, XBB.1.5, XBB.1.16, JN.1 and KP.3. We provide a detailed spatiotemporal description of the viral spread across main cerebral regions and the associated neuropathological alterations. Furthermore, brain transcriptomic analysis in susceptible K18-hACE2 mice infected with the ancestral Wuhan strain and the BA.1 VoC revealed distinct molecular pathways involved in the cerebral response to different SARS-CoV-2 VoCs. Our findings highlight key cellular and molecular brain alterations that may contribute to the neurological symptomatology observed during SARS-CoV-2 infection and long COVID-19 syndrome.

Neutrophil extracellular traps alter the long-term impairment of the glymphatic system and cognitive performance following thrombotic stroke

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Stroke is the second cause of death and a leading cause of chronic disability and cognitive deficits. During stroke, neutrophils generate neutrophil extracellular traps (NETs), which are related to a worsen outcome. The glymphatic system has a key role in the initial stages of stroke, first serving as a source of fluid contributing to the edema and, later, as an active clearer of the excess fluid in the brain. Emerging evidence suggests that stroke impairs the glymphatic system, but how NETs impact glymphatic activity is unknown. This study explores the short and long-term effects of NETosis on the glymphatic system using a mouse stroke model.

Wild type, DNase1-/-DNase1/3-/- and PAD4-/- male and female C57BL/6 mice underwent the permanent occlusion of the middle cerebral artery using the FeCl3 model. Cognitive and sensorimotor function was evaluated two months after stroke with behavioural tests. The glymphatic activity was assessed via cisterna magna injection of a 66,5KDa fluorescent tracer, and tracer coverage of brain sections and cervical lymph nodes was measured.

Our data shows that NETosis was present in meninges 24h after stroke and that glymphatic influx into the peri-infarct and glymphatic efflux to the lymphatic system was reduced in WT and DNase1-/-DNase1/3-/- compared to PAD4-/- ($p<0.05$, n=4-6). Two months later, PAD4-/- mice showed better sensorimotor function in the corner test, and improved learning in the Barnes maze compared to WT and DNase1-/-DNase1/3-/- mice ($p<0.05$, n=8-14). In addition, we observed a significant reduction in the glymphatic activity in the dorsolateral cortex in the DNase1-/-DNase1/3-/- mice (compared to WT) in the chronic phase of stroke ($p<0.05$, n=6-8).

This work points out that NETosis impacts the glymphatic system activity shortly after stroke and the effects are persistent in the chronic phase of stroke, both in the glymphatic activity and in the cognitive and sensorimotor outcome.

NMDA RECEPTOR-BK CHANNEL FUNCTIONAL COUPLING IS ALTERED IN THE FRAGILE X SYNDROME MOUSE MODEL

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The large conductance Ca2+ and voltage-activated K+ channel (BK) is widely expressed throughout the central nervous system and plays a critical role in diverse neuronal functions. BK channel activation is dependent on the synergistic action of cell membrane depolarization and increased in intracellular Ca2+ concentration, which is supplied by calcium sources located in close proximity to the channel. N-methyl-D-aspartate receptors (NMDAR) have been described as a functional Ca2+ source for BK channels. The study of these complexes was originally focused on hippocampal and cortical neurons. In the latter, we have proposed that NMDAR-BK multichannel complexes serve as high-pass filters for incoming synaptic inputs (Gomez et al., 2021). Nonetheless, growing evidence (including work from our laboratory) demonstrates that this mechanism is present in diverse neuronal types and may have been overlooked in many physiological settings (we have reviewed this topic recently in Martinez-Lazaro et al., 2025). Our previous work demonstrated that postsynaptic NMDAR-BK coupling in a BC-L5PN subpopulation mediates a negative feedback mechanism, conferring upon these neurons the ability to act as high-pass filters of incoming inputs, with filtering properties determined by the number and frequency of afferent stimuli. Here, we extend these findings by reporting functional NMDAR-BK coupling in additional brain regions, using a range of biophysical, cellular, and imaging techniques in brain slices. Notably, we also report significant disruptions of this coupling in a Fragile X Syndrome mouse model.

Non-HLA angiotensin-type-1 receptor autoantibodies mediate the long-term loss of grafted neurons in Parkinson's disease models

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Grafted dopaminergic precursors can integrate into the host tissue and alleviate motor symptoms in Parkinson's disease (PD). In some patients, deterioration of graft function occurred several months after observing a graft-derived functional improvement. Rejection of peripheral organs was initially related to HLA-specific antibodies. However, the role of non-HLA antibodies is now considered also relevant. Angiotensin-II type-1 receptor autoantibodies (AT1-AA) act as agonists of the AT1 receptors. AT1-AA are the non-HLA antibodies most widely associated with graft dysfunction or rejection after transplantation. However, it is not known about the presence and possible functional effects of AT1-AA in dopaminergic grafts, and the effects of treatment with AT1 receptor blockers (ARBs) such as candesartan on graft survival.
In a PD rat model, we studied the short-term (10 days)- and long-term (3 months) effects of chronic treatment with candesartan on the survival of grafted dopaminergic neurons, and the effects of dopaminergic denervation and grafting on serum and CSF AT1-AA levels. At the early period post-grafting, the number of grafted dopaminergic neurons that survived was not significantly different between treated and untreated hosts, probably because, just after grafting, other deleterious factors are predominant for dopaminergic cell death, such as mechanical trauma, lack of growth factors/nutrients and ischemia. However, several months post-grafting, we observed a significantly higher number of surviving dopaminergic neurons and a higher density of striatal dopaminergic terminals in the candesartan-treated group. For several months, grafted rats showed blood and cerebrospinal fluid levels of AT1-AA higher than normal controls, and also higher AT1-AA levels than non-grafted parkinsonian rats.
The results suggest using ARBs such as candesartan in PD patients, particularly before and after dopaminergic grafts, and the need to monitor AT1-AA levels in PD patients, particularly those candidates for dopaminergic grafting.
Funding: AEI (PID2021-126848NB-I00; PLEC2022-009401; PID2022-137079NB-100); ISCIII (RD21/0017/0031; PI20/00345, CIBERNED), XUGA (ED431C 2022/41), FEDER.

Novel insights in an experimental model of Post-Finasteride Syndrome: behavioral alterations and their molecular basis

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Finasteride, an inhibitor of the enzyme 5α-reductase, is commonly used to treat benign prostatic hyperplasia and androgenetic alopecia. Notably, patients treated with finasteride reported side effects related to sexual, psychiatric, neurological, and physical domains, that, in some cases, persist even after the drug discontinuation, leading to the so-called post-finasteride syndrome (PFS). The etiopathogenesis of this condition has been poorly explored. Therefore, we performed behavioral and molecular analyses after subchronic finasteride administration (i.e., 20 days) and its withdrawal (i.e., 1 month) in adult male rats. In particular, we studied the locomotor activity, anxiety-like behavior and the willingness to explore in a new environment by the open field test (OF) and curiosity by the novelty-seeking test (NST). Moreover, a genome-wide analysis by RNA sequencing in the hippocampus was performed in order to explore possible pathways involved in the PFS etiopathogenesis8. Finally, based on these results, we further investigate by immunohistochemistry one of the dysregulated pathways (i.e., the synaptogenesis). From a behavioral perspective, our data show increased locomotor activity and escaping of the anxiogenic zone after the suspension of finasteride by the OF and less curiosity, interest, and avoidance in visiting the novel added object into the arena by NST, despite no variations being found after subchronic finasteride treatment. RNA sequencing analysis revealed 19 differentially expressed genes in the hippocampus after the chronic treatment. The analysis at the drug withdrawal failed to identify dysregulated genes; however, Gene-Set Enrichment Analysis (GSEA) indicated that several gene sets were enriched at both time points. Some of the dysregulated genes and GSEA suggest a potential alteration in the synaptogenesis, which was confirmed by our immunohistochemistry studies. Altogether, these findings suggest a potential link with specific side effects previously observed in PFS patients, such as depression, anxiety, and disturbance in attention. (Supported by Post-Finasteride Foundation).

Oculomotor nuclei are resistant to ALS-induced changes in the expression of KCC2 and NKCC1 cotransporters

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ALS is a neurodegenerative disease characterized by progressive degeneration of motoneurons, leading of skeletal muscles atrophy and impaired voluntary motor function. However, not all motoneurons are equally vulnerable. Thus, extraocular motoneurons exhibit selective resistance, surviving even at later stages of the disease. It has been described that hyperexcitability of motoneurons is a typical feature of ALS that courses with a disturbance in the excitatory/inhibitory synaptic balance. In this context, it is important to highlight that reduced levels of KCC2 and increased levels of NKCC1 have been linked with neurological disorders characterized by hyperexcitability. KCC2 and NKCC1 are the primary cotransporters involved in chloride homeostasis in neurons, which directly affects the polarity of inhibitory neurotransmitters and, consequently, the excitatory/inhibitory synaptic balance and hyperexcitability.
Based on this, we aimed to compare the expression levels of these proteins between ALS-resistant (oculomotor, trochlear and abducens) versus ALS-vulnerable (facial and hypoglossal) motoneurons, throughout disease progression, using the Tg-SOD1/G93A mice as an experimental model of ALS. Mice were fixed intracardially, and the brainstem was cut into 30 µm-thick coronal sections. Triple immunofluorescence staining was performed for ChAT (to identify motoneurons), KCC2, and NKCC1, followed by confocal microscopy analysis.
Our results showed that the expression levels of KCC2 and NKCC1 proteins in the oculomotor nuclei of Tg-SOD1/G93A mice remained like those found in healthy littermate controls. In contrast, large changes were detected in the facial and hypoglossal nuclei of ALS mice, where KCC2 levels decreased and NKCC1 increased significantly in the symptomatic phase of the disease. This dysregulation of chloride cotransporters in vulnerable motoneurons may contribute to hyperexcitability in ALS and play a role in the etiology of the disease.

OPCs' inflammatory memory as a key factor in remyelination

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Oligodendrocyte Progenitor Cells (OPCs) are the main drivers for central nervous system remyelination. Upon demyelination, OPCs migrate to the damaged area, proliferate and differentiate into oligodendrocytes that regenerate the lost myelin, preventing axon degeneration and functional impairment. Successful remyelination relies on a controlled inflammatory response. However, chronic inflammation like that observed in multiple sclerosis (MS) and aging hinders OPC remyelination capacity. Recent studies have demonstrated that some tissue-specific stem cells can undergo inflammation-driven long lasting epigenetic reprogramming that modifies their response to subsequent stimuli. This phenomenon is known as inflammatory memory.

Our work investigated the short- and long-term impact of inflammatory stimulus on OPC biology to explore whether OPCs retain an inflammatory memory that modifies their response to demyelinating insults. To address this question, we are investigating OPC response to acute lipopolysaccharide (LPS)-mediated systemic inflammation in vivo. Transcriptomic analysis of OPCs 24 hours after LPS treatment revealed an increased expression of several immune- and disease-associated genes. 5 weeks after LPS injection, OPCs revert to their homeostatic state, and no longer express disease-associated markers. Notably, ATAC sequencing data coupled with super-resolution microscopy showed a persistently altered chromatin accessibility profile in OPCs 5 weeks after LPS treatment.

Our results reveal enduring epigenetic reprogramming of OPCs after LPS injection, which may represent OPC inflammatory memory. However, whether this reprogramming alters OPC response to subsequent stimuli and their regenerative properties is yet to be elucidated. Our ongoing and future work aims to understand how inflammatory memory influences OPCs response to demyelination. Exploring the role of OPC inflammatory memory and the underlying molecular pathways will provide new insights into remyelination failure in chronic inflammatory conditions, such as MS and aging. Altogether, this will help to developing new therapeutic approaches aimed at boosting remyelination.

Optimization and characterization of focal cortical stroke models using two-photon imaging

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Brain stroke is one of the most devastating conditions, often resulting in long-term disability for those affected. Despite its impact, no effective treatments currently exist for the sub-acute and chronic phase of the disease. Therefore, there is a need to focus on new techniques to better understand the processes that occur after brain stroke.

Focal stroke models were characterized using a glass implant for long-term two-photon imaging. Stroke models were induced via ligation of one of the middle cerebral artery branches which irrigate the right somatosensory cortex. Two approaches were developed: permanent stroke (pStroke), where the perfusion is not restored, and transient stroke (tStroke), where the perfusion is restored after 60 minutes.

Stroke injury was almost completely established within 6 hours of stroke onset in animals with pStroke, whereas in animals subjected to tStroke, a peak was observed at 3 days, as observed by cresyl violet staining and fluorojade staining. Likewise, pStroke mice exhibited a peak in glial reactivity at 24 hours, while tStroke mice displayed this peak at 3 days and sustained hyper-reactivity for a longer period. Ex vivo blood brain barrier permeability with Evans Blue injections showed a peak at 6 hours in pStroke mice, whereas tStroke mice reached the peak at 3 days. Regarding vessel structure, pStroke animals displayed a pattern of vessel growth at 15 and 30 days, while tStroke animals showed less vessel growth over time as shown by two-photon imaging. Moreover, vascular architecture showed higher complexity in pStroke compared to tStroke mice. Finally, behavioural tests results revealed similar sensory-cognitive deficits in both stroke models, although these deficits persisted longer in tStroke mice.

Our results suggest a novel approach for studying brain ischemia in live animals, enabling real-time hemodynamic analysis. While pStroke mice primarily exhibited primary ischemic damage, tStroke mice showed secondary damage.

Osteocalcin drives a hippocampal pro-autophagic profile to maintain cognitive resilience

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Systemic factors play a crucial role in maintaining postmitotic cells, such as neurons, and consequently support neuronal function. Neuronal aging is closely linked to hormonal imbalance, which contributes to age-related memory decline. However, the mechanisms through which hormones regulate and maintain neuronal function, and how neurons integrate hormonal stimuli from the extracellular milieu, remains poorly understood. Among the hormones that decline with age, the bone-derived pro-youth factor osteocalcin (Ocn) is drastically reduced. Using an unbiased proteomics approach, we found that Ocn injection into the hippocampus induced changes in acetylation-related proteins, suggesting that the acetylation/deacetylation pathway may be a potential mediator of its anti-aging effects. Sirtuin (SIRTs) deacetylase enzymes, particularly SIRT1, play a pivotal role in integrating metabolic and systemic factors into cells, thereby supporting neuronal homeostasis by modifying epigenetic marks throughout the lifespan. Thus, we sought to investigate whether SIRT1 mediates the pro-youth effects of Ocn on cognitive resilience through epigenetic mechanisms. First, we observed that Ocn and its receptor, Gpr158, activate SIRT1 and increase its nuclear levels, which is accompanied by a reduction in acetyl-H3k9 levels. Furthermore, we found that Ocn modulates H3k9 acetylation to regulate different transcriptomic pathways via SIRT1. Network analysis indicated that autophagy-related genes were among the most affected by Ocn. By genetic and pharmacological approaches, we observed that SIRT1 is essential for memory and autophagy machinery maintenance. Conversely, restoring SIRT1 activity in the aged hippocampus ameliorated age-related cognitive decline in an autophagy-dependent manner. Altogether, our data demonstrate that Ocn induces a pro-autophagy epigenetic program through SIRT1 activation, offering a potential therapeutic avenue to ameliorate age-associated cognitive decline.

Pathophysiological role of the endocytic adaptor AP-2 in developmental and epileptic encephalopathies

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Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of brain disorders emerging in early childhood that pronounce in epileptic seizures and cognitive, behavioral and neurological alterations which in most cases result linked to intellectual dissabilited and/or autism spectrum disorder. Causes of DEEs are often genetic changes. Pathogenic variants in genes encoding voltage- and ligand-gated ion channels are the most common ones. Advances in genomic techniques have allowed the discovery of additional genes encoding proteins involved in synaptic, regulatory, and developmental functions. The identification of a de novo mutation affecting the µ-subunit of the clathrin adaptor protein 2 (AP-2) in patients with DEE has established a novel link between neurodevelopmental phenotypes and a process most fundamental to cellular homeostasis, metabolism, membrane remodeling and signal transduction: clathrin-mediated endocytosis (CME), the main mechanism for cellular uptake and internalization of receptors from the plasma membrane. Hence, we have focused our studies in understanding whether the synaptic impairment caused by the pathogenic variant of AP-2 results from dysfunction in neuronal development or in neuronal maintenance or both. Our results uncover a role for CME in the maintenance of astrocytic intracellular ion homeostasis which is crucial for effective glutamate uptake. We have found that deficient endocytosis in glial cells leads to axonal pathology due to an overactivation of glutamate receptors as a result of decreased clearance of glutamate by the glial glutamate transporters as a consequence of altered ion homeostasis. In addition, neurons exhibit functional exo-endocytic impairment. These findings reveal a novel non-cell-autonomous mechanism underlying neurodegeneration, which will open new pharmacological perspectives to alleviate the aberrant synaptic phenotype underlying the patients suffering of DEEs.

Perinatal Disruption of NMDA Receptors with Ketamine Induces Novel Long-Term Autism-Related Phenotypes

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Autism Spectrum Disorder (ASD) and schizophrenia (SCZ) are neurodevelopmental disorders with distinct clinical profiles yet substantial overlap in symptomatology, including social impairments, cognitive deficits, and abnormalities in sensory processing. A growing body of evidence suggests that an imbalance in excitation and inhibition (E/I) plays a crucial role in both disorders driven, at least partially, by NMDA receptor (NMDAr) hypofunction and dysfunction in GABAergic interneurons, particularly these parvalbumin-positive (PV+). Additionally, perineuronal nets (PNNs), specialized extracellular matrix (ECM) structures involved in various forms of plasticity, are also implicated in these pathophysiological processes. While the NMDAr hypofunction theory has been extensively studied in SCZ, its role in ASD is less explored despite its potential to model core autistic traits. Thus, this study examined the effects of NMDAr hypofunction using a neurodevelopmental mouse model in which ketamine (30 mg/kg) was administered on postnatal days 7, 9, and 11 to conduct a brain-wide analysis of PV+ interneurons and PNNs in key ASD-associated brain regions and explore their link to previously undescribed ASD-phenotypes. In adulthood, the model exhibited novel ASD-like traits, including deficits in social behavior, cognitive flexibility, and motor and sensory processing, as well as disruptions in PV+ cells in the caudate-putamen, CA2 region, and somatosensory cortex, accompanied by alterations in PNNs. Notably, the cerebellum was also affected, displaying a severely disrupted E/I balance. Our findings support ketamine-induced NMDAr hypofunction as a translational model for ASD, emphasizing the impact of early developmental disruptions in neurological disorders. Moreover, the study highlights the significance of underexplored brain structures in ASD, such as PNNs, offering novel insights into their potential roles in neurodevelopmental disorders and opening new research directions.

Peripheral myeloid-derived suppressor cells: A new tool to predict optic nerve myelin damage in an experimental model of multiple sclerosis

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Multiple sclerosis (MS) is a highly heterogeneous neurodegenerative disease, demanding targeted therapies, reliable biomarkers, and validated quantification methods to enhance and assess remyelination. The optic nerve (ON) has been proposed as a relevant pathway for remyelination monitoring through visual evoked potentials. However, the relationship between ON damage and clinical severity in MS remains elusive. Our group has characterized monocytic myeloid-derived suppressor cells (M-MDSCs) as biomarkers of relapse recovery and disease severity in MS and its model experimental autoimmune encephalomyelitis (EAE). In the current work, we investigate the relationship between EAE severity and ON histopathological impairment and evaluate M-MDSCs as predictive biomarkers of ON myelin alteration.
A longitudinal individualized study of EAE progression was carried out for 35 days post-immunization (dpi), once clinical recovery was achieved. M-MDSCs were quantified in the peripheral blood of mice at disease onset and peak. Severity and recovery indices were determined for each mouse, enabling their classification as severe/mild and poor/good-recoverers. ONs were dissected out and CASPR immunostaining was performed in order to analyze Ranvier's node density, i.e. myelin integrity. Age/sex matched non-immunized mice (CT) were used for comparison. EAE mice at peak exhibited lower ON node density than CT, independent of prior disease severity. At 35 dpi, good-recoverers showed higher ON node density than those with a poor recovery, although neither group reached CT levels. Higher M-MDSCs levels at onset and peak correlated with enriched ON node density at both peak and recovery, respectively. ROC curve analysis revealed that M-MDSCs cut-offs of 4.50% at onset and 5.24% at peak predicted high ON node density at peak and recovery (70% and 90% specificity, respectively).

These findings highlight a strong connection between lower EAE disease severity, better clinical recovery, and preserved ON myelin. Additionally, M-MDSCs show potential for predicting ON myelin damage throughout the EAE disease progression.

Potential regulators of adult neurogenesis through glial transdifferentiation in Drosophila

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Adult neurogenesis is a crucial yet poorly understood process in the context of central nervous system (CNS) regeneration. In an adult CNS injury model in Drosophila, previously developed by our group to investigate glial responses following CNS insults, we described a novel mechanism by which glial cells transdifferentiate into neurons. This transdifferentiation phenomenon, significantly enhanced under injury conditions, represents a groundbreaking advance in the field of regenerative neuroscience.
Previous injury models in larvae identified the gene kon-tiki (kon) as a potential regulator of the glial regenerative response (GRR). Kon is a Drosophila homologue of NG2, a glial marker associated with repair processes in vertebrates, but its role in adult neurogenesis remains unexplored. Similarly, Glial Cells Missing (Gcm), the master regulator of gliogenesis in Drosophila, is associated with neurogenesis in certain contexts. Thus, these two factors could be involved in glial plasticity and transdifferentiation during CNS regeneration. Here we investigate the possible role of Kon and Gcm in this new mechanism of neurogenesis.

Pre-amyloid cognitive stimulation protects TgF344-AD rats against Alzheimer's disease pathological offenses on a multilevel scale

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Cognitive reserve is a mechanism of brain resilience that allows for a better cognitive performance than expected given a certain degree of disease, injury or brain changes associated with age. In the context of Alzheimer's Disease, it grants a better ability to cope with the disease's pathological insults. The objective of this study is to assess whether cognitive stimulation had a protective effect against Alzheimer's disease at a multilevel-scale (functional brain connectivity, neuroinflammation and synaptic plasticity markers).
The experimental design included a longitudinal study in male and female wildtype rats and the TgF344-AD Alzheimer's Disease rat model. Cognitive stimulation was conducted by periodically performing the Delayed Non-Match to Sample task starting from an early age (3 months), before amyloid deposition begins in the model. Longitudinal magnetic resonance imaging (MRI) acquisitions were performed and processed to assess training-induced changes in brain connectivity over time. Memory performance was assessed using the novel object recognition test. Synaptic plasticity markers were assessed, as well as neuroinflammation-related data, to provide a mechanistic explanation of the protective effects of the training.
Cognitive stimulation exerted a protective effect on aged rats, as the model animals maintained their functional connectome and showed a preservation of memory in male TgF344AD rats. Additionally, cognitive stimulation enhanced the expression of plasticity-related proteins in the model rats and transiently preserved a wild-type-like phenotype in microglial cells
Our results suggest that cognitive stimulation enhances biological pathways related to synaptic plasticity and promotes a less reactive microglial phenotype. This allows for a large-scale networks reconfiguration and increases the resilience against Alzheimer's disease-induced memory deficits

Profiling the human precuneal proteome across neuropathological staging in Alzheimer´s disease: Sex-dependent effects and computational repositioning of antineoplastics drugs

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Alzheimer's disease (AD) is the most common cause of dementia, affecting men and women in a significantly different way. In the present work we have performed sex-dependent proteomics across AD Braak staging in the precuneus, a region mainly involved in spatially guided behavior and episodic memory retrieval.
Label-free LC-MS/MS were applied in 32 post-mortem precuneal specimens classified as: non-demented (n = 4M / 4W); Braak stage I-II (n = 5M / 3W); Braak stage III-IV (n = 4M / 4W); and Braak stage V-VI (n = 4M / 4W). In addition, drug-repositioning workflows and in vitro experiments were performed.
From 6261 identified precuneal proteins, 131 differential expressed proteins (DEPs) were detected in Braak stages I-II, 94 DEPs in Braak stages III-IV and 141 DEPs in Braak stages V-VI, suggesting that the severity of the proteostatic imbalance across neuropathological staging was practically similar. Interestingly, a conjoint evaluation of neuropathological and sex dimensions revealed that AD women showed the highest number of DEPs in Braak I-II (251 DEPs), whereas men's Braak I-II has an intermediate level of DEPs (76 DEPs), pointing out that different precuneal protein expression trajectories exist between sexes across Braak staging. Using a computational tool for drug repurposing, two compounds with capacity to reverse early AD-precuneal omics signatures were in vitro validated, demonstrating neuroprotective effects against hydrogen peroxide or tau prefibril-induced neurotoxicity.
In conclusion, sex-dependent alteration of precuneal proteostasis during AD progression was evidenced. Protein expression changes were more abundant in women than men regardless of neurological staging. Our data pointed out that precuneal omic signatures may be useful as substrates for drug repurposing in AD. Ongoing experiments in animal models will help us to validate these potential compounds, proposing novel therapeutic approaches to be used in AD treatment.

Protein Palmitoilation in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of the nigrostriatal pathway, which is essential for motor control. This degeneration leads to symptoms such as resting tremor, rigidity, and bradykinesia.
Protein palmitoylation (PP), a reversible lipid post-translational modification (PTM), regulates protein trafficking and membrane association. Previous studies on the human cerebral cortex have revealed significant differences in the palmitome (PP proteome) between PD patients and healthy individuals. However, very few studies have investigated PP-related changes associated with PD and their functional effects.
In this study, we examined the expression and PP levels of PD-associated proteins in skin fibroblasts and plasma from sporadic PD patients and age- and sex-matched healthy controls. We performed ABE palmitoylation assays followed by western blots and proteomics studies.
Our study suggests, for the first time, the potential palmitoylation of PD-associated proteins LRRK2, OPA1, GCasa1, UCHL1, and fibrinogen α, β, and γ, and confirms previous findings indicating the palmitoylation of DJ1. These results indicate that plasma and skin fibroblasts are suitable samples for detecting alterations in the palmitome of PD patients. Notably, the accessibility of these samples facilitates the study of pathological mechanisms and the future development of biomarkers. Furthermore, the reversibility of this PTM offers the possibility of exploring PP as a potential therapeutic target for developing disease-modifying treatments.

PROTEOMIC INTERROGATION OF THE NUCLEUS BASALIS OF MEYNERT DURING ALZHEIMER'S DISEASE

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The Nucleus basalis of Meynert (NbM) is considered the main center of cholinergic innervation of the cerebral cortex. Cholinergic neurons in the NbM integrate information from subcortical regions and project it to the frontal cortex, hippocampus, or amygdala. NbM plays an indispensable role in the subcortical regulation of memory and attention, in which cholinergic abnormalities occur as early as asymptomatic or at prodromal stages of AD. Moreover, recent evidence suggests that neuropathological changes of the entorhinal cortex are preceded by degeneration of the NbM in AD models. However, the intrinsic molecular imbalance that occurs at the level of the NbM during AD progression is largely unknown.
Label-free quantitative (phospho)proteomics were applied in 52 post-mortem NbM derived from controls with no known neurological history and AD subjects. Moreover, drug-repositioning workflows and in vitro experiments were performed.
Proteome and phosphorylation-based signal transduction imbalances were observed at the level of NbM in AD. Analysis considering the sex variable showed: i) protein expression and signaling differences in control women and men NbM's, ii) 50% more differential expressed proteins in AD women than in AD men, iii) a different functional profile across sexes and iv) the NbM phosphorylation is more severe in AD men than AD women. Using a computational drug repurposing workflow, we found natural compounds and drugs with capacity to in silico reverse early AD-related omics signatures. In-vitro validation assays revealed that the pretreatment of cholinergic neurons with a natural compound or a chemical drug had a neuroprotective effect against amyloid-beta and tau-induced neurotoxicity. Further combinatorial assays will help us to elucidate the therapeutic potential of both compounds against AD.

Regional differences in dendritic spine morphology and excitatory input in hippocampal and entorhinal cortex regions of an Alzheimer's disease mouse model

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Alzheimer's disease (AD) is characterized by cognitive deficits and synaptic dysfunction. The hippocampal trisynaptic circuit, which receives afferent connections from the entorhinal cortex (EC) and includes the dentate gyrus, and the hippocampal CA3 and CA1 regions, plays a critical role in learning and memory. Within this circuit, dendritic spines, which vary in shape and size, are crucial for synaptic plasticity and function. This study examined dendritic spine morphology and density in the apical and basal dendrites of principal neurons in the CA1, CA3, and EC regions of wild-type (WT) mice and a murine model overexpressing GSK-3β. Intracellular injections of Lucifer yellow were used for spine visualization, and excitatory input was assessed using VGLUT1 labeling. In WT mice, apical and basal dendrites showed distinct spine distributions. CA3 basal dendrites exhibited a reduced density of thin spines, while EC dendrites showed increased density and a higher percentage of mushroom spines. In the AD model, region-specific alterations included reduced spine density, a higher percentage of thin spines and fewer mushroom spines in both apical and basal CA1 dendrites. CA3 basal dendrites displayed higher density of mushroom and stubby spines. In the EC, spines in both dendritic domains had smaller heads, and basal dendrites showed fewer density and percentage of mushroom spines. Additionally, a significant decrease in excitatory input was observed in apical and basal CA1 dendrites and in basal CA3 dendrites in the AD model. These findings suggest a shift from mature mushroom spines to immature thin spines, along with a reduction in excitatory synapses in specific hippocampal regions, pointing to disrupted synaptic plasticity and stability. These alterations may lead to synaptic impairments, halted long-term potentiation, and cognitive decline. The study emphasizes the importance of understanding regional differences in dendritic spine alterations, highlighting a selective impact of AD on distinct hippocampal areas.

Regional functional connectivity in aging networks

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Aging is associated with profound changes in brain structure and cognitive function, yet its impact on the brain's functional organization remains poorly understood. Emerging research suggests that aging affects networks, which are thought to drive neurodegenerative disease progression (Chauveau et al., 2025; Seeley et al., 2009; Vogel et al., 2020). Much of our understanding of how these networks change with age comes from studies on functional connectivity (FC) in resting-state networks (RSN), using resting-state functional magnetic resonance imaging (rs-fMRI). However, while functional connectivity in younger populations is well characterized (Jockwitz & Casper, 2021), regional connectivity patterns in older adults remain unclear. This inconsistency arises partly due to small sample sizes and a predominant focus on global rather than regional FC changes within RSNs. This study examined how aging modifies patterns of regional functional connectivity using 3,179 rs-fMRI sessions from 960 participants (ages 43–97) in the OASIS-3 dataset. Imaging data were preprocessed using the Human Connectome Project pipeline, and resting-state networks were identified via group spatial Independent Component Analysis (ICA). Regional FC changes were assessed using linear mixed-effects models, accounting for age, clinical status and episodic memory. Findings revealed significant age-related reductions in FC across six RSN, however the magnitude was neither uniform across nor within networks. While some networks exhibited widespread declines, others demonstrated a heterogeneous pattern of both regional increases and decreases. The balance between decreases and increases shifted across age groups as reductions appeared earlier and more robustly, whereas increases developed later and gradually. These imbalances were also associated with clinical status and episodic memory performance. Taken together, these findings suggest that aging induces network-specific changes in functional brain dynamics, potentially contributing to cognitive decline and progression toward clinical dementia. These insights enhance understanding of FC changes and may inform new diagnostic and therapeutic strategies for early-stage dementia.

Rehabilitation strategies shape motor and sensory outcomes after cervical spinal cord injury

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Spinal cord injury (SCI) leads to severe motor and sensory impairments, often accompanied by neuropathic pain, which affects more than 40% of patients and significantly reduces their quality of life. While activity-based rehabilitation is the only intervention proven to promote motor recovery, its impact on different functional domains remains unclear. Additionally, pain management remains challenging, as current treatments are often insufficient or have significant side effects. Evidence suggests that rehabilitation engages a widespread network of spinal and supraspinal structures, but different rehabilitative approaches may exert distinct effects on motor recovery and pain modulation. This study aimed to compare two rehabilitative strategies and evaluate their effects on motor function and pain perception.

C57Bl6/J mice underwent an incomplete C5 spinal cord transection, followed by eight weeks of either task-specific directed rehabilitation, free unspecific rehabilitation in an enriched environment, or no rehabilitation. Rehabilitation started one week post-injury. Motor performance (CatWalk, horizontal ladder, Whishaw's test, cylinder test) and pain perception (tail immersion, Von Frey) were assessed at 1, 3, 5, and 9 weeks post-injury. At the end of the study, spinal cord sections were stained with cresyl violet and luxol blue to characterize lesion extent and identify affected spinal tracts.

Both rehabilitative strategies, particularly task-specific training, improved gross forelimb motor function. However, none of the approaches restored fine manual dexterity. Pain outcomes were also improved in rehabilitated mice compared to non-rehabilitated ones, with enriched environment rehabilitation specifically reducing paraesthesias.

These findings reinforce the benefits of activity-based interventions in SCI recovery and highlight that different rehabilitation strategies may differentially impact motor and sensory outcomes. However, their limitations emphasize the need for complementary neuromodulatory strategies to optimize functional recovery.

Relationship between cognitive ability and cardiovascular function after physical exercise in older adults

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The progressive aging of the population has led to an increase in the prevalence of diseases in which age constitutes the primary risk factor, as is the case with dementia. Specifically, Alzheimer's disease is the most common type of dementia. Moreover, this disease is characterized by a prolonged prodromal phase, which complicates early diagnosis and effective treatment. Therefore, a better understanding of the mechanisms that drive the onset and progression of neurodegeneration in the early stages of the disease could represent a promising approach in the search for preventive strategies and new therapeutic alternatives.
In this study, we evaluated cardiorespiratory regulation capacity and its relationship with physiological cognitive decline in individuals over 60 years of age. To achieve this, participants underwent a cognitive assessment using the Mini-Mental State Examination (MMSE) and subsequently performed an intense 5-minute walk. Following physical activity, we monitored blood pressure, heart rate, and peripheral temperature for 15 minutes.
Our results indicate that physiological aging induces significant changes in cardiorespiratory function and cognitive abilities. In our study, the limited cardiovascular adaptation to physical activity was dependent on the degree of cognitive preservation.
Furthermore, individuals with lower cognitive agility exhibited difficulties in regulating peripheral temperature, whereas those with better cognitive preservation demonstrated a more efficient cardiovascular response and adequate peripheral thermal regulation after exercise. These findings suggest that maintaining optimal cardiorespiratory function contributes to preventing cognitive decline associated with aging, thereby reducing the risk of developing dementias.
Funding: Proyectos de investigación UGR/Ciudad Autónoma de Ceuta 2024-Ref: CE-06-UGR24

Repercussions of Myelin Deficit on Neural Progenitors in the absence of R-Ras1 and/or R-Ras2

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Previous studies from our laboratory have demonstrated that R-Ras1 and R-Ras2 are essential in myelination processes within the central nervous system. The absence of these GTPases blocks the differentiation of specific oligodendrocyte subpopulations, leading to a myelin deficit associated with an increase in the number of immature oligodendrocytes.
Therefore, mice lacking R-Ras1 and/or R-Ras2 proteins exhibit varying degrees of hypomyelination. This myelin deficit induces mitochondrial dysfunction, astrogliosis and microgliosis, which lead to axonal loss and degeneration. Ultimately, these processes lead to a loss of neuronal function in the nervous system.
In parallel, it has been observed that the adult central nervous system maintains a reservoir of undifferentiated oligodendrocyte progenitor cells, intended to replenish dying oligodendrocytes and ensure remyelination. These progenitors play a crucial role in regeneration and repair following injuries or damage, and their ability to differentiate into mature oligodendrocytes is fundamental for myelin restoration. However, these repair dynamics are altered in the hypomyelinated brains of mice lacking R-Ras1 and/or R-Ras2 proteins.
In this study, we present the repercussions of the myelin deficit on neural progenitors from mice lacking R-Ras1 and/or R-Ras2, providing a new perspective on how the myelin deficit affects adult brain repair dynamics and opening new opportunities for the study of therapies that could enhance remyelination in demyelinating diseases.

Restoring Microglial Reactivity and Phagocytosis Through IGF-1 Intervention

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Microglial activation is crucial in the neuroinflammatory response following traumatic brain injury (TBI). Aging leads to microglial dysfunction and decreased IGF-1 expression, impairing recovery. We examined the effect of IGF-1 gene therapy on microglial response in an aged TBI model.
Male and female mice received intramuscular injections in the right hindlimb of adenoviral vectors carrying either IGF-1 cDNA (RAd-IGF-1) or DsRed (RAd-DsRed). Two weeks later, animals underwent TBI and were sacrificed 7 days post-injury. Microglial myelin phagocytic capacity was assessed via immunocytochemistry by quantifying dMBP puncta colocalized with IBA1-positive cells. Primary microglial cultures from P2 mice were maintained for 2 or 16 days to represent young and aged cells, stimulated with CSF-1 and treated with IGF-1. We measured mRNA expression of IL-6, IL-1β, TNFα, MCP1 (CCL2), CCR2, P2RY6, TREM2, and MSR1 to evaluate inflammatory and phagocytic signaling. In addition, phagocytic capacity was evaluated by measuring the fluorescence of neuronal debris or bioparticles in cells stimulated with IFNγ.
In vitro, CSF-1 increased inflammatory gene expression (IL-6, IL-1β, TNFα) regardless of sex or age, with IL-6 showing the highest change. IGF-1 alone did not alter these levels, but cotreatment reduced CSF-1-induced IL-1β and TNFα expression, notably in aged female cells. In terms of phagocytosis, P2RY6 and MCP1 increased more in aged cells and IGF-1 enhanced phagocytic gene expression. Primary microglial cultures incubated with IFNγ showed no significant phagocytic differences compared to controls, consistent with senescence; however, adding IGF-1 in the presence of IFNγ increased neuronal debris phagocytosis and decreased bioparticle uptake.
In vivo, saline-treated aged animals had fewer, larger IBA1-positive cells near the lesion compared to IGF-1-treated animals, which showed more dMBP puncta inside microglia. Additionally, female animals exhibited lower dMBP puncta regardless of treatment.
These data indicate that IGF-1 significantly modulates microglial morphology, reactivity, and phagocytosis.

RNA-seq analysis of gene expression and splicing in mice carrying two Knock In (KI) mutations in the Tardbp/TDP-43 gene

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TDP-43, encoded by the TARDBP gene, is an RNA-binding protein that plays an important role in regulating mRNA processing and splicing. The dysregulation of its function has been linked to the development of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases.
In this study, we examine two KI mouse models carrying point mutations in the endogenous Tardbp: M323K and Q331K. Both mutations affect de C-terminal domain of TDP-43. The Q331K mutation has been associated with ALS, and although the M323K mutation has not been specifically linked to the disease in humans, it has shown to cause a gain of function effect in RNA splicing and progressive motor neuron degeneration in mice.
To understand how these mutations impact RNA metabolism, we performed RNA sequencing (RNA-seq) on spinal cord samples from both strains of mice at 3 and 18 months of age. By comparing mutant and wild type mice, we aim to identify age-related changes in gene expression and alternative splicing that may contribute to neurodegeneration, and their potential role in ALS pathology.

Role of Cortistatin in Neuroimmune Communication During Aging

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Aging is a complex, progressive, and irreversible process that significantly impacts the immune system (IS) and the central nervous system (CNS), leading to chronic diseases with high personal and social costs. Few preclinical models reflect the neuroimmune dysregulation associated with aging, limiting our understanding of its molecular bases, and hindering the identification of common mediators between IS and CNS. Previous studies suggest that cortistatin, an anti-inflammatory neuropeptide produced by both systems with specific receptors, may regulate IS-CNS communication. Furthermore, there is a correlation between the decrease in cortistatin levels, systemic immune activation, and neurodegeneration. However, the effect of cortistatin in neuroendocrine regulation during aging remains unknown. In this study, we characterized the role of cortistatin in IS-CNS interaction during aging dynamics. We used wild-type and cortistatin-deficient animals to analyze both biological niches (IS and CNS), their activation under physiological conditions, and the transition to pathological states. Additionally, we identified differentially expressed molecular pathways associated with the lack of cortistatin during aging. Our results demonstrated that cortistatin deficiency leads to premature aging, characterized by a reactive pro-inflammatory phenotype in both the peripheral and central systems. We observed molecular and functional alterations in the absence of cortistatin, affecting pathways involved in memory and learning regulation, synaptic processes, myelination, and metabolism. Overall, our findings indicate that cortistatin plays a key role in immune dysregulation during aging and highlight the interaction between the immune and nervous systems in the development and progression of CNS pathologies associated with aging. Notably, systemic and local variations in cortistatin levels, along with their association with neurodegenerative diseases suggest that cortistatin could be considered a biomarker and part of the molecular signature for early identification of these diseases.
Funded by the Junta de Andalucía PROYEXCEL_00867 and the FPI program (PRE2018-084824; MCIN/AEI /10.13039/501100011033/ and FEDER “A way of making Europe”).

Role of one-carbon metabolism in corticospinal neuron development in mouse models of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) mostly manifests during adulthood, implicitly suggesting that it hits the fully mature central nervous system. However, the evidence of a long prodromal phase, the existence of juvenile forms of ALS, and the developmental roles of genes implicated in familial cases suggest that ALS may take its root during development. Interestingly, neurodevelopmental and neurodegenerative diseases share several mechanisms, including alterations in one-carbon metabolism (1Cmet). 1Cmet intertwines the folate and methionine cycles, is controlled by four key enzymes (DHFR, MTHFR, MAT2A and AHCY), and is central to purine synthesis and cellular proliferation, and to methylation and epigenetic regulation. Here, we test the hypothesis that ALS subtypes might arise from neurodevelopmental 1Cmet impairments, focusing on the disease-vulnerable corticospinal neurons (CSN) in the Sod1G86R and Fus+/ΔNLS mouse models of ALS. Rresults indicate that Dhfr expression is significantly increased in E13.5 cortical progenitors (CPs) of both ALS mouse models, when they generate CSN. Ongoing analyses will confirm these data via DHFR enzymatic activity measurements and LC-MS assessment of 1Cmet metabolites. To test whether altered Dhfr expression may impinge on cellular proliferation, we performed BrdU labelling, DNA content analysis on purified CPs, and immunofluorescence on CPs and post-mitotic neurons. This allowed us to rule out impaired proliferation, suggesting that altered Dhfr expression might affect other cellular mechanisms, such as epigenetic regulation of gene expression. We thus purified E13.5 CPs from Sod1G86R and Fus+/ΔNLS embryos and conducted Cut&Tag analyses on H3K4me3 and H3K27me3. Work is in progress to reveal the epigenetic and transcriptomic landscape of CPs as they generate CSN and to evaluate the consequences on CSN function and survival. As 1Cmet strongly relies on diet, this project may not only inform on the consequences of 1Cmet alterations in familial cases but also in sporadic ALS cases.

Role of TACR3 and Testosterone in Anxiety-Related Synaptic Plasticity

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Pubertal failure and anxiety are common symptoms in individuals with dysfunctional TACR3, yet the underlying mechanisms remain unclear. This study explored the relationship between TACR3 expression, anxiety, sex hormones, and synaptic plasticity. Rats were assessed for anxiety-like behavior using the elevated plus maze. Severe anxiety was associated with reduced TACR3 expression in the ventral hippocampus. In female rats, TACR3 expression fluctuated across the estrous cycle, indicating hormonal sensitivity. During sexual development, hippocampal TACR3 expression increased alongside elevated serum testosterone levels, coinciding with reduced anxiety. A reciprocal regulation between hippocampal TACR3 and testosterone was observed.
To explore TACR3's role in synaptic plasticity, its activity was pharmacologically inhibited, leading to hyperactivation of the PKC pathway, CaMKII activation (Western blot), enhanced AMPA receptor phosphorylation (FORTIS), and increased spine density, analyzed via Imaris software. Functional consequences were assessed using a multielectrode array (MEA), revealing stronger cross-correlations of neuronal firing. TACR3-deficient rats exhibited lower testosterone levels, increased spine density in the dentate gyrus, and impaired long-term potentiation (LTP), recorded via electrophysiology.
Overexpression of functional TACR3 via Sindbis virus resulted in spine shrinkage and pruning, whereas the expression of defective TACR3 led to increased spine density, size, and cross-correlation magnitude. Neurons expressing defective TACR3 displayed abnormal firing responses to LTP induction, which were restored by testosterone treatment. These findings provide insights into the intricate relationship among TACR3, sex hormones, anxiety, and synaptic plasticity, offering potential therapeutic targets for TACR3-related anxiety disorders.

Role of thrombospondin-1 in blood-brain barrier protection after traumatic brain injury

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Traumatic brain injury (TBI) is the second most common cause of acquired brain damage in adults. It is estimated that in Spain, there are a total of 420,000 people with this type of injury, with an incidence of 100,000 new cases each year. TBI is caused by an external force, and its consequences can have a wide-ranging impact on the quality of life of affected individuals.

Previous findings from our group demonstrated that the matricellular protein thrombospondin-1 (TSP-1) plays a key role in maintaining the blood-brain barrier (BBB) after TBI in our closed-skull TBI animal model. However, patient serum data revealed an inverse correlation: lower TSP-1 levels were associated with better outcomes six months post-injury. Therefore, we decided to investigate the proteolysis process of TSP-1, focusing on the potential protective effect of its N-terminal fragment.

In in vitro studies with murine brain microvascular endothelial cells showed increased eNOS phosphorylation upon treatment with the N-terminal fragment. Additionally, in vitro vessel formation in Matrigel experiments revealed that the fragment significantly enhances segments formation. This protective effect was studied in our TBI animal model, where animals treated with the N-terminal fragment exhibited reduced BBB disruption.

Additionally, considering the severe vascular impairment observed in TSP-1 knockout (KO) animals after TBI, we employed Light Sheet microscopy to analyse their baseline vascular architecture. Our findings suggest that TSP-1 regulation involves proteolysis, highlighting the importance of further research to elucidate this mechanism and its therapeutic implications in TBI recovery.

This study is funded by Fundación Mutua Madrileña, by Fondo de Investigaciones Sanitarias (FIS) (ISCIII/FEDER) (PI22/00362) by Ministerio de Ciencia, Innovación y Universidades (Consolidación Investigadora CNS2023-145023).

R-Ras1 and R-Ras2 regulate mature oligodendrocyte subpopulations

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In the mammalian central nervous system, axonal myelination, executed by mature oligodendrocytes (MOLs), enables rapid neural transmission. Conversely, myelin deficiencies are hallmark features of multiple sclerosis, optic neuromyelitis, and some leukodystrophies. Recent studies have highlighted that MOLs are heterogeneous; however, how MOL subpopulations are specified and balanced in physiological settings is poorly understood. Previous works have demonstrated an essential role of the small GTPases R-Ras1 and R-Ras2 in the survival and myelination of oligodendrocytes. In this study, we aimed to determine how R-Ras1 and R-Ras2 contribute to the heterogeneity of MOL subpopulations. Our results evidence that R-Ras1 and R-Ras2 affect specification into the distinct subpopulations MOL1, MOL2, and MOL5/6, which in turn vary in their dependence of these GTPases. In R-Ras1 and/or R-Ras2 mutant mice, we observed an increase in MOL1 subpopulation and a decrease in the MOL2 and MOL5/6 subpopulations. We identified R-Ras1 and R-Ras2 as key elements in balancing the heterogeneity of MOLs. Our results contribute to the understanding of the molecular mechanisms underlying the heterogeneity of MOLs and the myelination processes, which is crucial for innovating regenerative therapies for nervous system disorders.

Sex Differences in APOE4-Driven Neuroinflammation in the Elderly: Challenging for Females and Life-Threatening for Males?

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The concept of inflammageing links chronic inflammation to biological aging, contributing to senescence and multimorbidity commonly observed in the elderly. Neurodegenerative diseases, such as Alzheimer's disease, exhibit significant inflammatory components associated with cognitive and physical decline. Moreover, accumulating evidence suggests that inflammatory processes may play a causal role in accelerating neurodegeneration. Apolipoprotein E (APOE), particularly the e4 allele, is a major genetic risk factor for neurodegeneration, acting as a key immunomodulator. This becomes even more relevant given the lack of prevention strategies for APOE ε4 variant carriers.
Our objective was to characterize the APOE4 genotype response under a chronic inflammatory challenge in aged transgenic mice. Male and female mice homozygous for humanized apoE4 between 15 and 16 months of age were administered intraperitoneal doses of either 0 or 0.25mg/kg of lipopolysaccharide (LPS) thrice a week on alternate days for a total of 17 doses (over 6 weeks). Animals were sacrificed 24h after the last injection and tissues were collected. Body weight was monitored throughout the experiment. Hippocampal and serum markers were assessed for inflammation, oxidative stress, myelination and neuroplasticity.
Our findings revealed pronounced sex-dependent differences in inflammatory response under LPS challenge. Females showed a greater response to the inflammatory stimuli, potentially leading to harmful outcomes in brain. Conversely, males showed a distinct vulnerability profile, with a lower-grade immune response but a higher mortality rate, suggesting altered immune responsiveness. Overall, our results underscore a multifaceted vulnerability scheme, emphasizing APOE genotype and sex as critical risk factors and encouraging new paths for future interventions.

Sex-associated clinical severity is related to the peripheral immune profile in experimental multiple sclerosis

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The incidence of multiple sclerosis (MS) is higher in woman than in men, with male sex being a poor prognostic factor for the disease. Sex-related immune differences and their potential impact on clinical severity remain under-investigated. These differences could be crucial for advancing personalized biomarker research and developing sex-specific immune-based treatments. The objectives of this study are: i) to compare the severity of the clinical course of experimental autoimmune encephalomyelitis (EAE) in males/females; ii) to study the relationship between sex-specific immune variations and their association with differences in clinical severity. To address these questions, an EAE model was developed in 8-week-old C57BL/6 mice of both sexes. Individualized daily clinical follow-up yielded several clinical parameters, and peripheral blood samples were collected individually at the onset and peak of each animal. Flow cytometry analysis was performed to assess monocytic myeloid-derived suppressor cells (M-MDSCs), neutrophils, classical dendritic cells (cDCs) and activated T cells. Our results showed that males had an earlier onset and more severe clinical course than females, mainly related to less clinical recovery. Furthermore, male peripheral blood showed lower M-MDSC and cDCs abundance, and higher neutrophils than females at both onset and peak. Importantly, M-MDSCs in males at peak displayed a more differentiated profile than in females. Regardless of sex, M-MDSCs abundance at peak was related to less severity. Males also had a higher percentage of activated T lymphocytes at onset and a higher percentage of CD8+ T cells at all time points analyzed. In sum, male M-MDSCs are scarcer than in females and seem to exert compromised immunosuppressive activity due to their over-differentiated phenotype. The enrichment of activated lymphocytes and neutrophils in males at the peak of EAE may indicate a greater tissue damage. Overall, sex-specific immunological differences are consistent with the worsened symptom recovery observed in males versus females.

Sex-dependent progression in the 5XFAD model of Alzheimer's disease: insights from synaptic, behavioral, and spatial proteomics analyses

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In the early stages of Alzheimer's disease (AD), hippocampal dysfunction emerges as a prominent neuropathological hallmark, primarily linked to the accumulation of amyloid-β (Aβ) peptide oligomers. In the 5xFAD mouse model of AD, characterized by the overexpression of mutant forms of amyloid precursor protein (APP) and presenilin-1 (PS1), hippocampal dysfunction initiates around 2-3 months, coinciding with the early deposition of Aβ plaques. While previous studies have explored this dysfunction, a significant gap remains in understanding its progression and sex differences at behavioral, synaptic, and molecular/proteomic levels.
In this study, we characterized the sex-dependent progression of AD in 3- and 6-month-old 5xFAD mice across multiple levels: (1) Synaptic function was assessed through CA3-CA1 long-term potentiation (LTP) and paired-pulse facilitation (PPF) in hippocampal slices. (2) Behavioral impairments were evaluated using the object location memory (OLM) task for hippocampal-dependent spatial memory, along with the elevated plus maze (EPM) and tail suspension test (TST) to assess anxiety- and depression-like behaviors. (3) Spatial proteomic alterations were examined using MALDI imaging mass spectrometry to analyze differential protein expression and spatial distribution within the hippocampus.

Our results reveal distinct sex differences in disease progression. At 3 months, males exhibited significant deficits across all levels, while females remained unaffected. By 6 months, impairments began to manifest in females, whereas in males, these deficits persisted or worsened. These findings highlight the importance of considering sex as a critical factor in disease progression and underscore the need for sex-specific therapeutic strategies for AD.

MCIN/AEI/10.13039/501100011033 (PID2020-115823-GBI00), JCCM and ERDF (SBPLY/21/180501/000150) and UCLM/ERDF (2022-GRIN-34354) to JDNL and LJD; predoctoral fellowship JCCM and ERDF (SBPLY/23/180502/000032) to JMF.

Sexual dimorphism in morphological and functional alterations of a mouse model of Parkinson's disease overexpressing A53T α-synuclein

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Parkinson's disease (PD) is characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the accumulation of α-synuclein (α-syn) aggregates. Beyond α-syn aggregation, dysregulation of autophagic and endo-lysosomal trafficking may be implicated in disease progression, although precise mechanisms remain unclear. Notably, PD exhibits sexual dimorphism in disease onset, progression, and treatment response. This study aimed to investigate the morphological and functional changes in a PD mouse model overexpressing α-syn, particularly focusing on sex differences.
Male and female C57BL/6J mice received bilateral intranigral injections of AAV vectors encoding mutant human A53T α-syn or empty vectors. At 120 days post-surgery, motor function was assessed with Open Field, Wire Hang and Balance Beam tests. Mice were sacrificed and immunohistochemical analyses were performed to evaluate α-syn pathology and nigrostriatal degeneration (tyrosine hydroxylase, TH). Additionally, autophagosomal and lysosomal activity was assessed via immunofluorescence staining for LC3 and LAMP1.
α-syn overexpression resulted in motor impairments in male but not female mice, as evidenced by prolonged beam-crossing time and reduced mean event walking bout duration in the Balance Beam test. These behavioral deficits were associated with a significant reduction in TH immunoreactivity in the striatum and a subtle, non-significant decrease in SNc dopaminergic neuron density. Despite the reduction in striatal TH positivity, α-syn-overexpressing male mice also exhibited a greater abundance of enlarged axonal swellings in the striatum. Although α-syn overexpression activated the autophagic-lysosomal system in both sexes, only male mice demonstrated increased autophagic and lysosomal capacity with enhanced autophagic flux.
These findings highlight significant sex differences in motor symptom onset, neurodegeneration, and autophagic response, underscoring the need for sex-specific therapeutic approaches in PD treatment.

Funding: Grant PID2 021-126434OB-I00 funded by MCIN/AEI/10.13039/501100011033, ERDF A way of making Europe; Basque Government (IT1706-22, PUE21-03); Transborder Joint Laboratory (LTC) "non-motor Comorbidities in Parkinson's Disease (CoMorPD)".

SFRP1: A new potential therapeutic target for Alzheimer's Disease

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Alzheimer's Disease (AD) is a neurodegenerative disorder with a high socio-economic burden. While the origins of AD are still under debate, current treatments targeting amyloid-beta (Aβ) plaques and toxic peptides remain largely ineffective, highlighting the need for novel therapeutic strategies. Previous work from our lab described Secreted Frizzled-related protein 1 (SFRP1) as a novel player in AD pathogenesis, establishing it as a promising target. SFRP1, a glia-derived protein that accumulates in the brains and cerebrospinal fluid of AD patients, negatively regulates ADAM10, a protease involved in amyloid precursor protein (APP) processing, neuroinflammation, and synaptic function. High SFRP1 levels promote amyloidogenic APP cleavage, stabilize toxic Aβ conformations, foster neuroinflammation, and disrupt synaptic plasticity. To target SFRP1, we developed a highly specific monoclonal antibody (mAb 10.5.6) with strong neutralizing activity. In APP;PS1 mice, retro-orbital (RO) administration of 10.5.6 counteracts AD pathogenic traits by reducing Aβ plaques and neuroinflammation, and preventing cognitive decline, compared to non-specific IgG treated controls. For characterizing the immunotherapeutic properties of 10.5.6, we compared different routes of administration and tracked the body distribution of biotinylated and radiolabeled forms of the mAb in APP;PS1 mice. We first observed that RO injections, as compared to intraperitoneal, resulted in higher levels of 10.5.6 in the bloodstream, enabling it to reach the brain, with no apparent peripheral toxicity. We moved on to treat APP;PS1 mice with different dosages (100, 200 µg) starting at different stages of the disease (2, 4, 9 and 12 months of age). Our findings show an effective decrease of amyloid burden and neuronal dystrophy exclusively in animals that are treated with higher doses or at the onset of the disease. This study paves the way for innovative disease-modifying therapies, offering a more comprehensive understanding of AD pathology.

Signature-driven repurposing of HDAC1 and PARP-1 inhibitors in Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by progressive motor, including tremor, rigidity, and bradykinesia, alongside cognitive decline, and non-motor dysfunction, such as olfactory impairment. Despite significant progress in understanding the molecular and cellular events associated with PD, there remains an unmet clinical need for effective therapies. In this study, proteomic analysis of the olfactory tract (OT) in controls with no known neurological history (n=17) and PD subjects (n=21) revealed Lewy body disease (LBD) stage-dependent proteostatic impairment, accompanied by progressive modulation of alpha-synuclein (α-syn) interactome. We implemented a computational drug repositioning approach from PD-related brainstem omics signatures (BOSs), evaluating OT differential PD datasets as well as nine differential transcriptomics data from PD-affected brainstem regions such as substantia nigra, putamen, striatum, locus coeruleus and cortex. Although this analysis did not reveal global disease-modifying compounds, HDAC-1 and PARP-1 inhibitors were identified as the top drug candidates with in silico potential to restore multiple BOSs. To explore the potential therapeutic effects of the selected drugs, in vitro assays were performed using olfactory and dopaminergic neuronal cell lines treated with α-syn preformed fibrils (PFFs). These validation experiments revealed that pre-treatment with an HDAC-1 or a PARP-1 inhibitor reduced neurotoxicity induced by α-syn PFFs and resulted in a neuroprotective effect against oxidative stress-induced damage. These findings confirm the suitability of omics profiles in drug repositioning workflows against PD, offering valuable insights into the beneficial potential of HDAC1 and PARP-1 inhibitors in the therapeutic pipeline of PD.

Slow release of synthetic RAR agonists from chitosan nanoparticles for the therapy of spinal cord injury

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Spinal cord injury (SCI) frequently causes sensitive and/or motor dysfunctions with devastating personal and social consequences and significant economic costs to society. Strategies aimed at obtaining functional recovery based on axonal regeneration after SCI have so far not been successful. Lesioned neurons activate a program of axonal regeneration, but this fails in the microenvironment of the injured spinal cord.
Our strategy consists in using novel synthetic retinoic acid receptor (RAR) agonists that have shown to promote axonal regeneration even in the presence of growth inhibitory stimuli. These compounds, specifically EC23 and NVG0645, were developed by Nevrargenics Ltd for therapeutic applications in the CNS. To be effective after SCI, we consider a prolonged and local activation of RAR as necessary. For this we have established a methodology for the slow pharmacological release from chitosan nanoparticles, with the intention to inject these at the site of SCI.
Chitosan nanoparticles were prepared and loaded with RAR agonists, their release and biological effects were tested in vitro. Here, we present data on drug incorporation and release from nanoparticles, half-life of RAR agonists under culture conditions and their effects on RARβ-dependent gene expression. The results provide a basis for the local sustained drug application after SCI.

Spatial relationship between CD44+ astrocytes and wasteosomes

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Wasteosomes, also referred to as corpora amylacea, are spherical polyglucosan bodies that accumulate in the periventricular, perivascular, and subpial regions of the human brain, particularly during aging and in various neurodegenerative diseases. Recent research suggests that wasteosomes are originated in astrocytes, and act as waste containers that remove waste products from the brain and may be involved in a cleaning process of the central nervous system. However, the mechanisms driving their formation and development remain unknown.

In this work, we analysed brain tissue from five patients, including four with Alzheimer's disease and one with Pick's disease. Our study focused on sections of the hippocampus and the superior and medial temporal lobe. We performed immunofluorescence staining using antibodies against GFAP to identify astrocytes, CD44 to detect interlaminar astrocytes, and p62 as a specific marker for wasteosomes. We examined the distribution and localization of these markers within the tissue, aiming to identify potential colocalization patterns that could provide insights into their interactions.

We have confirmed the presence of wasteosomes in subpial regions of the temporal lobe, particularly near the Sylvian fissure, as well as in subpial areas of the hippocampus, such as the hippocampal sulcus and the fimbriodentate sulcus, and in periventricular regions. Moreover, we observed that, in the areas where wasteosomes accumulate, astrocytes are CD44+.

This spatial correlation suggests a potential relationship between the formation of wasteosomes and the interlaminar astrocytes in the brain interfaces. Further investigation is needed to elucidate the functional significance of this co-distribution and its potential role in neuropathological conditions.

Spatial Transcriptomic Profiling of the human dorsal striatum in Parkinson's disease Using Xenium In Situ Sequencing

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The dorsal striatum, a critical component of the basal ganglia, integrates cortical inputs to regulate motor and cognitive functions. In Parkinson's disease (PD), a neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra, striatal dysfunction arises from dopamine depletion, leading to both motor and cognitive deficits. Despite its central role in PD pathophysiology, the striatum's spatial transcriptomic landscape and its disease-associated alterations remain poorly resolved.

Spatial context is essential for unraveling tissue complexity, as molecular interactions are governed by cellular organization within anatomical niches. In situ sequencing (ISS) technologies, such as the Xenium platform, enable high-resolution spatial mapping of transcripts while preserving tissue architecture, offering unprecedented insights into cellular heterogeneity and microenvironmental dynamics.

Here, we applied Xenium ISS to post-mortem human caudate and putamen tissues from PD patients and healthy controls. Our study achieved single-cell resolution transcriptomic profiling while retaining spatial information, revealing the striatum's cellular diversity and supracellular organizational principles. We identified 34 distinct cell types grouped into four main classes, as well as spatially defined "domains" characterized by unique cellular compositions, reflecting functional subregions. Comparative analysis uncovered PD-associated transcriptomic signatures. Expression gene module analysis highlighted two divergent pathways enriched on the disease: An unfolded protein response (UPR) and a neuroinflammatory program, exhibiting significant negative correlation between each other. Neuronal populations, particularly medium spiny neurons (MSNs), displayed distinct dysregulation patterns, suggesting compartmentalized vulnerability.

These findings elucidate the striatum's spatial architecture and delineate PD-specific perturbations across cellular compartments, advancing our understanding of PD progression and the disease's impact on this brain region.

Spatially profiling the proteome of Alzheimer's disease vulnerable neurons in vivo

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Selective neuronal vulnerability is a characteristic of many neurodegenerative disorders. In Alzheimer's disease (AD), the first neurons to degenerate are neurons in the entorhinal cortex layer II (ECII). Despite their relevance in the disease's development and progression, what makes these neurons more susceptible to AD pathology remains unknown, and represents one of the biggest challenges in AD. While cell-specific transcriptomics have helped describe the early alterations in AD, mRNA levels lack the essential information about protein expression and function at a subcellular level. Here we developed a system to build up our knowledge of ECII neuron biology by using cell-specific, spatially resolved proteomics in vivo. We utilised TurboID, a novel biotin ligase to design a system that allows proteomic analysis with subcellular resolution. We have generated AAVs expressing TurboID fused either to a nuclear export sequence or synaptophysin, to target the somatodendritical and presynaptic compartments of ECII neurons respectively. The expression of these constructs is dependent on a neuron-specific promoter. In this project, we explore how major AD drivers trigger alterations in the subproteome of ECII neurons. To study how the proteome of ECII neurons is affected by amyloid pathology, we use APP NL-F knock-in mice, a mouse model of AD that expresses the amyloid precursor protein with two pathogenic mutations, the Swedish (NL) and the Iberian (F). These mice develop amyloid plaques from 6 months of age, and cognitive impairment at 18 months. We have characterised the proteome of 3-month-old APP NL-F mice, before the development of plaques, to detect early changes in ECII neurons.
Results from this study will identify key proteins that contribute to AD pathology, paving the way to discover novel therapeutic targets for the disease.

Spermidine, a novel treatment for GRIN2B-related disorder

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GRIN-related disorders (GRD) developmental encephalopathies are associated with a clinical spectrum including developmental delay, hypotonia, intellectual disability, epilepsy, and autistic traits. The presence of pathogenic de novo GRIN gene variants results in the disturbance of the N-methyl D-aspartate receptor (NMDAR) function in a gene- and residue-dependent manner. Despite recent advances to elucidate GRD pathophysiological mechanisms and to find treatments, to date GRD therapeutic arms are still scarce and with limited efficacy.

Herein we investigated whether the natural polyamine spermine -positive allosteric modulators of GluN2B subunit-containing NMDARs- or its precursor spermidine might rescue NMDAR hypofunctionality. In heterologous cell systems, administration of spermine potentiated wildtype and loss-of-function (LoF) NMDAR-mediated currents and attenuated synaptic density deficits. Functionally, the putative therapeutic benefit of spermidine (spermine precursor) was assessed in constitutive Grin2b+/- haploinsufficient mice, a GRIN2B-LoF genetic murine model recapitulating GRD-like synaptic, motor, and cognitive alterations. Chronic spermidine administration in young adult Grin2b+/- mice partially rescued hippocampal long-term potentiation deficits in hippocampal slices of Grin2b+/- mice, supporting the cognitive improvement observed in behavioural phenotyping.

Based on these preclinical findings, a case study was conducted in two paediatric patients harbouring mild GRIN2B-LoF variants. Importantly, in line with preclinical findings, 18-months spermidine treatment resulted in the amelioration of adaptive behaviour measured by Vineland Adaptive Behaviour Scale, with the absence of noticeable side effects. Overall, our findings provide both preclinical and clinical data supporting the benefit of spermidine for the treatment of GRD in individuals harbouring GRIN2B-LoF variants.

Statins, Angiotensin Type-1 Antagonists and ROCK Inhibitors Reduce L-DOPA Induced Dyskinesia in a Rat Model of Parkinson's disease

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Statins have been proposed for L-DOPA-induced dyskinesia (LID) treatment. Statin anti-dyskinetic effects were related to the inhibition of the Ras-ERK pathway. However, the mechanisms responsible for the anti-LID effect are unclear. Changes in cholesterol homeostasis and oxidative stress- and inflammation-related mechanisms such as angiotensin II and Rho-kinase (ROCK) inhibition may be involved. The nigra and striatum of dyskinetic rats showed increased levels of cholesterol, ROCK, and the inflammatory marker IL-1β, which were reduced by the angiotensin type-1 receptor (AT1) antagonist candesartan, simvastatin, and the ROCK inhibitor fasudil. As observed for LID, angiotensin II-induced, via AT1, increased levels of cholesterol and ROCK in the rat nigra and striatum. In cultured dopaminergic neurons, angiotensin II increased cholesterol biosynthesis and cholesterol efflux without changes in cholesterol uptake. In astrocytes, angiotensin induced an increase in cholesterol uptake, decrease in biosynthesis, and no change in cholesterol efflux, suggesting a neuronal accumulation of cholesterol that is reduced via transfer to astrocytes. Our data suggest mutual interactions between angiotensin/AT1, cholesterol, and ROCK pathways in LID, which are attenuated by the corresponding inhibitors. Interestingly, these three drugs have also been suggested as neuroprotective treatments against Parkinson's disease. Therefore, they may reduce dyskinesia and the progression of the disease using common mechanisms.
Funding: Spanish Ministry of Science and Innovation (grant numbers: PID2021-126848NB-I00, PLEC2022-009401), Spanish Ministry of Health (grant numbers: RD21/0017/0031, PI20/00345, and CIBERNED), Galician Government (XUGA, gran number ED431C 2022/41) and FEDER (Regional European Development Fund).
Keywords: L-DOPA; Parkinson; Rho-Kinase; angiotensin; brain cholesterol; dyskinesia; fasudil; neuroinflammation; oxidative stress; simvastatin.

STIMULATION OF CORTICOSPINAL NEURONS BY OPTOGENETIC cAMP INDUCTIONS PROMOTES MOTOR RECOVERY AFTER SPINAL CORD INJURY VIA RAPHESPINAL TRACT MODULATION

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After spinal cord injury (SCI), cAMP levels significantly drop in the spinal cord, the sensorimotor cortex, and the brainstem compromising the CNS regenerative potential. To address SCI recovery, we delivered cAMP by the overexpression of the bacterial photoactivatable adenylate cyclase (bPAC) for on-demand corticospinal neurons activation. Motor cortex daily optogenetic stimulation increased P-CREB and c-Fos expression, increased passive membrane excitability, and restored the firing threshold and action potential firing of the spinal cord injured animals close to the healthy levels. Six days after stimulation and SCI, bPAC treated group significantly recovered fine locomotor skills without affecting nociception. Retrogradely traced descending neurons from below the injury, showed an increased number of neurons at the motor cortex and the raphe-reticular formation but not in the red nuclei, demonstrating the greater regeneration of the corticospinal and raphe/reticulospinal tracts through the injury site after bPAC treatment. Accordingly, a higher number of 5-hydroxytryptamine positive descending serotoninergic axons caudal to the injury were found in bPAC-stimulated rats. The serotonergic descending pathways implication in motor recovery was further evidenced by selective depletion of the serotonergic neurons (via 5,7-dihydroxytryptamine injections) resulting in the abrogation of bPAC-mediated functional recovery. Overall, our findings underscore the efficacy of the solely bPAC-stimulated cAMP induction in corticospinal neurons in recovering function after SCI, which allowed us to describe a cortical rerouting pathway via the serotonergic descending tract.

Stroke-Induced Immunosuppression: Differential Roles of the SNS and HPA Axis in Lymphopenia

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Stroke is a leading cause of mortality and disability worldwide, resulting from a sudden interruption of blood flow to a specific brain region. Beyond its direct neurological consequences, stroke profoundly impacts the immune system, triggering an initial pro-inflammatory response followed within hours by systemic immunosuppression. This immunosuppression, characterized primarily by lymphopenia, increases susceptibility to infections and worsens patient outcomes.
Although activation of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis after stroke has been implicated in driving this immune suppression, the precise mechanisms underlying lymphocyte loss remain insufficiently understood. In this study, we used a transient middle cerebral artery occlusion mouse model and spectral flow cytometry to analyze immune cell populations three days post-stroke. To assess the role of SNS and HPA axis activation, we performed chemical sympathectomy with 6-OHDA and inhibited glucocorticoid signaling using RU486, a glucocorticoid receptor antagonist.
Our results show that post-stroke immunosuppression is marked by a significant reduction in T lymphocyte numbers across multiple organs, including the thymus, spleen, and liver. Notably, chemical sympathectomy effectively prevented lymphocyte loss in the spleen but not in the thymus. Similarly, blocking glucocorticoid signaling with RU486 mitigated immunosuppression in the spleen and had partial effects on thymic immune cells.
These findings suggest that while SNS and HPA axis interventions can prevent splenic immunosuppression, different immune organs respond uniquely to stroke-induced signals. The thymus appears to undergo lymphocyte depletion through mechanisms independent of SNS and glucocorticoid signaling, underscoring the need for further investigation. Understanding these differential immune responses could inform targeted therapeutic strategies to mitigate post-stroke immunosuppression and reduce infection-related complications in stroke patients.

Study of early alterations in lipid metabolism in the murine model of neuromuscular pathology SOD1G93A

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Amyotrophic lateral sclerosis (ALS) is a multifactorial and multistep fatal degenerative disorder. There are many unknowns in the disease aetiology of ALS. Both patients and animal models have significant metabolic dysregulation such as hypermetabolism, dyslipidemia and progressive weight loss. The origin of these lipid metabolic alterations remains unclear. We aim to identify early lipid metabolic pathways altered at the onset of disease (p90) in the spinal cord of mouse model of ALS. We performed a transcriptomic analysis of the spinal cord of SOD1G93A and wild type littermate mice at p90 and identified three main lipid pathways deregulated related to sphingolipids, eicosanoids and cholesterol metabolism. Alterations of cholesterol metabolism at spinal cord were validated by qPCRs where an inhibition of cholesterol synthesis and an increase in cholesterol transport and accumulation were observed in the initial stages of the pathology (p90). Even at presymptomatic stage (p60) we identified an upregulation of Ch25h gene. All these transcriptomical alterations were exacerbated in symptomatic stages of the disease (p120). Finally, to verify the importance of these alterations in lipid metabolism, an in-vivo study has been performed where a modulator of lipid metabolism has been administered from presymptomatic stage (p60) until the ethical end-point. SOD1G93A mice treated with this lipid modifier in the diet show an increase in the survival and improvement of grip strength, locomotor coordination (rotarod), muscle electrical response (EMG) at p120 when compared to SOD1G93A mice with control diet.

STUDY OF THE INTERACTION BETWEEN MICROGLIA AND ENDOTHELIAL CELLS IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Dementia is a term for several diseases that affect memory, thinking and daily activity performance. The most prevalent form of dementia is Alzheimer's disease (AD), which explains 60-70% of dementia cases. AD is a neurodegenerative disorder characterized by brain atrophy, amyloid β (Aβ) and tau proteinopathies, gliosis (astrocyte and microglia reactivity) and vascular dysfunction. Previously, our group has described that a non-productive angiogenesis phenomenon occurs around Aβ plaques, giving rise to disassembled low-perfused vascular structures, which we have called vascular scars (VaS). We have also observed that microglia are not only reactive to Aβ deposits, but also to nearby endothelial cells, which could contribute to the VaS formation. As microglia get exhausted during the progression of AD, we wonder whether the vascular dysfunction could be altered when the microglia activity is reduced in AD. With this aim, we are studying the vascular consequences of depleting microglial cells in an AD mouse model using an inhibitor of CSF1R, whose signalling is essential for microglia survival. Our results show an efficient microglial depletion, which has a potential effect on amyloid accumulation. Strikingly, we have not been able to completely deplete microglia around Aβ deposits, suggesting that they are hypoperfused structures as it has been previously described. We have also observed a reduction in Aβ associated vascular abnormalities along with a worsening in cognitive performance, prompting us to think that microglia have a vascular protective role. Altogether, our results highlight the relevance of an accurate interaction between microglia and endothelial cells in the Aβ context.

STUDY OF THE ROLE OF NLRP3 IN PRESERVING BLOOD-BRAIN BARRIER INTEGRITY IN ISCHEMIC STROKE

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Stroke is a leading cause of death and disability worldwide. Ischemic stroke, which accounts for 80–85% of all stroke cases, results from a reduction in blood flow to a specific brain region, leading to neuronal death and inflammation. A crucial consequence of this process is the disruption of the blood-brain barrier (BBB), impairing the functionality of the neurovascular unit, exacerbating brain damage, and worsening patient prognosis. A key player is the NLRP3 inflammasome, a multiprotein complex responsible for producing pro-inflammatory cytokines. While previous studies have demonstrated the involvement of NLRP3 in stroke, its specific role in distinct cell types and its impact on the neurovascular unit remain unclear.

This study aimed to investigate the interaction between pericytes and endothelial cells—two critical regulators of BBB permeability following cerebral ischemia—and to elucidate the role of NLRP3 in these cell populations. To achieve this, we assessed BBB disruption in vitro using a 3D co-culture of cells, subjected to hypoxia/reoxygenation to simulate cerebral ischemia. Additionally, we conducted an in vivo murine model of 1-hour transient middle cerebral artery occlusion (tMCAO) using an intraluminal filament to evaluate BBB integrity at 4 and 24 hours of reperfusion in wild-type (WT) mice, NLRP3 knockout (KO) mice, and post-stroke MCC950-treated animals.

In the in vivo model, WT mice showed significant BBB rupture at 4 and 24 h post-reperfusion. In KO mice, BBB rupture increased at 4 h but decreased at 24 h compared to WT, suggesting a dual role of NLRP3 over time. MCC950 treatment (30 min post-ischemia) protected the BBB. In the in vitro model, we are exploring the function of NLRP3 in each cell type.

In conclusion, NLRP3 plays a complex, tiem-dependent role in BBB integrity after brain ischemia, likely due to its different function in pericytes and endothelial cells.

SUBCELLULAR PROFILING REVEALS MITOCHONDRIAL ALTERATIONS IN REACTIVE ASTROCYTES OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a complex neurodegenerative condition that causes progressive memory loss and dementia. In AD brains astrocyte become reactive potentially contributing to cognitive decline. Astrocyte reactivity is a highly complex phenomenon with remarkable morphologic and molecular phenotype changes, and the role of astrocytes in the development of AD is still unknown. Astrocytes are the prevalent glial cells in the brain and have a large number of functions aimed at maintaining brain homeostasis including regulation of brain energy metabolism, maintenance of the blood-brain barrier, ion homeostasis, synaptic activity and plasticity, among many other functions. Any disruption regarding the normal roles of astrocytes can result in morphological and functional changes that ensue in pathological consequences. Mitochondrial dysfunction is an early event in the pathogenesis of AD, although most studies have focused on neurons and little is known about the functional characteristics and the dynamics of astrocyte mitochondria. We had performed an ultrastructural analysis using transmission electron microscopy in the hippocampus of amyloidogenic (APP/PS1) and tauopathy (P301S) mice. Our results show structural alterations in mitochondria that include double membrane rupture, cristae loss, and fragmentation together with a loss of their circularity. Since mitochondrial morphology is directly related to mitochondrial fusion/fission processes, the ultrastructural changes observed in astrocyte mitochondria in these amyloidogenic and tauopathy models suggest dynamic abnormalities in these organelles that may lead to deficits in astroglial function compromising their capability to maintain brain homeostasis and support neuronal energy metabolism and survival. A better understanding of cell type-specific mitochondrial dysfunction as a pathological feature of AD might hold great potential for the exploration of novel molecular targets for therapeutic development.

Supported by grants from Instituto de Salud Carlos III FIS PI24/00274 (AG) y PI24/00308 (JV), Fondos FEDER de la Unión Europea, Junta de Andalucía P18-RT-2233 (AG) y US-1262734 (JV), CIBERNED.

TAF1-dependent transcriptional dysregulation underlies multiple sclerosis

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Multiple sclerosis (MS) is characterized by neuroinflammation and demyelination of the central nervous system, leading to disability. Genetic variants that confer MS risk implicate genes involved in immune function, while variants related to severity are associated with genes preferentially expressed within the CNS. Current immune-related therapies fail to slow-down disease progression, and the molecular events that trigger progressive MS remain unknown. TAF1, a subunit of the general transcription factor TFIID that participates in RNA polymerase II (RNAPII) transcription initiation and promoter-proximal pausing, has been related to neurological diseases. Given that alteration of RNAPII promoter-proximal pausing in oligodendrocytes is associated with MS, we wondered whether TAF1 could have a role in MS pathogenesis.

We analyzed TAF1 levels and distribution in postmortem brains of individuals with progressive MS by Western blot and histology. Genetically modified mice recapitulating the MS patient TAF1 alteration were generated, which displayed a progressive MS-like phenotype, and were studied through RNA-seq, biochemical, histological and behavioral approaches. Through proteomics in an oligodendroglial cell line, ChIP-seq of RNAPII in mice and co-localization studies in human postmortem brains, we dissected the underlying molecular mechanism responsible for the MS-like phenotype.

We found underdetection of TAF1 C-terminal region in MS brain, attributable to endoproteolytic cleavage by extralysosomal cathepsin B. We demonstrated in genetically modified mice that C-terminal alteration of TAF1 suffices to induce changes in RNAPII promoter-proximal pausing affecting oligodendroglial myelination-related genes. This results in an MS-like transcriptomic signature accompanied by CNS-resident inflammation, robust demyelination and MS-like motor phenotype. Finally, we identified numerous interactors of C-terminal TAF1 that participate in RNAPII transcription, of which two (GTF2B and GTF2H4) show evidence for genetic association to MS. Therefore, TAF1 dysfunction converges with genetic susceptibility to cause MS-like transcriptional dysregulation. Our study reveals the first biochemical alteration in MS brain that suffices to trigger progressive MS.

Targeting stathmin family proteins in neurodegeneration: new insights from cellular model studies

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Neurodegenerative diseases (NDDs) are a group of disorders characterized by the progressive loss of neurons that result in motor and cognitive dysfunction and are a leading cause of disability and mortality in the population. Despite several attempts, the success of neuroprotective therapies remains limited by our incomplete understanding of the neurodegenerative process. Stathmin-2 (STMN2) is a member of the highly conserved family of proteins known as stathmins (STMNs). STMN2 is a key protein in microtubule regulation and axonal maintenance and has been identified as a potential therapeutic target in some NDDs. Our aim in this work is to explore the role of STMN2 and other STMNs family members in the process of neurodegeneration. We used human SH-SY5Y and murine Neuro-2a neuroblastoma-derived cell lines and exposed the cells to various stressors, including proteosome inhibitors (bortezomib), microtubule destabilizers (vincristine) and a compound that induces mitochondrial dysfunction (oligomycin). Subsequently, we evaluated the effects of these treatments on cell viability and analyzed the expression levels of STMN2 and other members of the STMNs family by RT-qPCR and Western blotting. The results indicated that vincristine reduces STMN2 at protein level without altering its mRNA expression, while increasing STMN4 mRNA expression levels. Notably, STMN2 overexpression was able to protect cells from oligomycin- and vincristine-induced death but failed to prevent cell death caused by thapsigargin. These findings highlight the relevance of STMN2 and STMN4 as potential therapeutic targets in NDDs associated with alterations in the microtubule cytoskeleton and mitochondria. However, further research is needed to better understand the mechanisms regulating their expression and to enhance their neuroprotective effect.

The activation of A1AR improves hippocampal synaptic and behavioural alterations in a mouse model of Fragile X Syndrome

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Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and a leading monogenic cause of autism spectrum disorder. It is caused by silencing the Fmr1 gene and the loss of expression of the Fragile X Messenger Ribonucleoprotein (FMRP). FMRP is an RNA-binding protein that negatively regulates the expression of postsynaptic and presynaptic proteins and its loss produces an increment in neurotransmitter release associated to two factors: the increase in the pool of synaptic vesicles in contact with synaptic membrane (docking) and ready to release (RRP), and the overactivation of voltage-gated calcium channels (VGCCs). FXS patients and the mouse model lacking FMRP (Fmr1KO) show multiple behavioural alterations, including deficits in memory acquisition and learning which there is currently no specific treatment.

We performed electron microscopy, whole-cell patch-clamp electrophysiology and behavioural experiments to characterise the synaptic mechanisms underlying the memory acquisition and learning deficits in both sexes Fmr1KO mice. Since it is well known that adenosine A1 receptors (A1AR) are located at the synaptic terminals of Schaffer collaterals (SC) where reduce glutamate release by inhibiting VGCCs, we also tested the efficiency of different agonists of these receptors as a therapeutic strategy for hippocampal alterations in FXS.
We found that significant increase in the docking of synaptic vesicles at SC-CA1 synapse of Fmr1KO which was associated with an enhancement the synaptic efficacy caused by an increase in release probability and RRP size. Interestingly, A1AR activation by its specific agonist CHA reverts these synaptic alterations to control levels. Moreover, intraperitoneal injection A1AR-permeable agonists significantly improves spatial memory deficits of Fmr1KO mice.

These results reveal that this therapeutic strategy, based on the reduction of calcium influx into the presynaptic terminal through A1AR activation, seems promising for the treatment of both synaptic and behavioural alterations in the Fmr1KO mouse model.

The cross-communication of neuromuscular junctions: not just a motor nerve-skeletal muscle talk

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Neuromuscular junctions (NMJs) play an essential role in muscle function, since they support synaptic connections between motor neurons and skeletal muscles. They are covered by terminal non-myelinated Schwann cells (tSCs), which are involved in NMJ maintenance and repair, and also by kranocytes, fibroblast-like cells with unknown functions. NMJs have been largely considered "isolated" structures. However, they are not just synaptic junctions, but a really complex microenvironment not completely understood as well. Through confocal microscopy, electron microscopy and scRNA-seq analysis, we have analysed these NMJ-capping cells in homeostasis, upon several injuries and in Amyotrophic Lateral Sclerosis disease. First, we observe that kranocytes are the key players in the communication of NMJs with their microenvironment, establishing contacts between adjacent NMJs and even contacting surrounding vascularization. Moreover, tSCs and kranocytes completely differ their response to stress conditions: tSCs strongly preserve their position at the NMJ while kranocytes rapidly react and change their conformation, even before motor nerve retraction. These structural adjustments also occur distally to the injure location. Finally, we demonstrate that NMJs are not such "isolated" structures, but a highly connected structure, always interacting with their microenvironment.

The impact of ischemic lesion on the cellular microenvironment and long-term neurobehavioral outcomes in a novel murine model of pediatric stroke

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Pediatric ischemic stroke is a cerebrovascular disease caused by the obstruction of a cerebral artery, occurring between day 29 of life and 18 years of age. Despite being a rare disease, pediatric stroke is a major cause of death and morbidity in children. However, little experimental data is addressing the underlying pathophysiology of pediatric stroke as few preclinical models are available.
Here, we present a novel model of pediatric stroke based on the transient occlusion of the distal middle cerebral artery (dMCA) in postnatal day 14-15 mice. The artery is exposed via craniotomy and compressed with a blunted capillary for sixty minutes to induce ischemia. To validate the model, infarct volume was assessed with 2,3,5-triphenyltetrazolium chloride at 1-day post-stroke (dPS) (n=7 animals). Furthermore, neuronal, microglial and astroglial densities were analyzed in the injured and control regions at 1dPS by multiple fluorescent labeling (n=4 animals per condition). Neurological deficits were evaluated by performing an adapted version of the SHIRPA battery, novel object recognition and open field tests at baseline, 1dPS, 3dPS, 7dPS, 14dPS (n=10 animals per condition).
The occlusion led to cerebral infarction and neuronal loss at 1dPS, which was reversed by 14dPS. At 1dPS, there was an increase in the surface area of neuropil occupied by astroglia and microglia within the ischemic regions, along with a higher cell density of these glial types. However, neurobehavioral testing indicated a minor effect of ischemia during the acute phase that was followed by a progressive improvement until near-complete recovery at 14dPS.
This study demonstrates that the newly developed pediatric model of focal transient ischemia accurately replicates the pathophysiology of ischemic brain injury. Consequently, it would be a valuable tool for future research into the early mechanisms of stroke-induced damage.

The Intracellular Domain of the Epilepsy Protein PCDH19 Regulates Spine Density in Cortical Neurons

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Mosaic mutations in the X-linked cell adhesion molecule Protocadherin 19 (PCDH19) lead to epilepsy with cognitive impairment, whereas complete absence of functional protein does not elicit any symptoms. It is believed that mosaic expression of PCDH19 leads to defective neuronal communication, but whether further roles beyond cell adhesion are critical for PCDH19 function in the cortex is currently unknown. We confirm proteolytic processing of PCDH19 in cortical neurons and show that nuclear transport of its intracellular domain is mediated by importins. RNAseq analysis further indicates that the intracellular domain of PCDH19 leads to broad transcriptomic changes. Finally, we use in utero electroporation to provide the first in vivo data about the role of this cleaved intracellular domain in upper layer cortical neurons, where it increases Xlr gene expression and reduces spine density without affecting overall dendritic morphology. Our results suggest that PCDH19 could act as an activity sensor in a synapse to nucleus signaling pathway involved in synaptic homeostasis.

The motor protein Miro1 is critical in the intercellular mitochondrial transfer to glioblastoma cells

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Glioblastoma (GBM) is the most common and lethal glioma, due to its high ability for tissue infiltration and chemoresistance. Recent discoveries reveal that GBM cells form tunneling nanotubes (TNTs) that communicate with surrounding neural cells, promoting GBM infiltration and tumor progression. TNTs enable GBM cells to establish coordinated networks and communications, ranging from intercellular signaling to the sharing of intact and functional mitochondria. In this context, the dynamics of mitochondrial transfer require active transport mediated by the motor adaptor Miro1, which is critical for the anterograde movement of organelles to the periphery of the donor cells. However, whether and how Miro1 orchestrates the transfer and integration of mitochondria into GBM cells remains unaddressed.

In this work, live microscopy, flow cytometry, and morphometric analysis show that, as expected, Miro1 is critical for the intercellular transfer of mitochondria to GBM cells. Genetic knockout models effectively block the anterograde transport of mitochondria, and we demonstrate for the first time that this process can be modulated by pharmacologically reducing Miro1 levels. As a result of Miro1 loss of function, mitochondria are absent inside TNTs, and mitochondrial transfer to GBM cells is drastically reduced, even though the number of TNTs remains unaltered. Notably, Miro1 appears to be irrelevant in acceptor cells to incorporate exogenous free mitochondria. On the other hand, donor cells lacking Miro1 may still release mitochondrial content, which can be captured by adjacent GBM cells.

In summary, our results place Miro1 as a key regulator of mitochondrial transfer via TNTs and highlight its importance in understanding the mechanisms governing this process in GBM progression. Importantly, the genetic and pharmacological modulation of Miro1 strongly endorses this protein as a potential therapeutic target to halt brain tumor development.

The neuroprotective effect of DdPAC light-stimulation on hippocampal astrocytes compared to neurons in Alzheimer's disease

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The pathophysiology of Alzheimer's disease (AD) is characterized by the accumulation of extracellular Amyloid-β (Aβ) plaques, neuroinflammation and neuronal dysfunction. Additionally, Aβ-plaques disrupt cyclic adenosine monophosphate (cAMP) levels and signalling, associated with impaired synaptic plasticity and memory formation. Here, we sought to determine whether selectively increasing cAMP in neurons or astrocytes within the hippocampus of 5xFAD mice could mitigate AD pathophysiological alterations. We used a red-light-sensitive photoactivated adenylate cyclase (PAC), DdPAC, to selectively elevate cAMP in either neurons or astrocytes upon illumination. AAV9 constructs expressing DdPAC or GFP under GFAP or CamKII promoters were injected in the hippocampal DG from 8-month-old 5xFAD or WT mice. After four weeks, red-light stimulation (λ 685 nm) was delivered in freely moving mice for 10 minutes (1 sec ON, 4 sec OFF). 24h after, tissue was processed for immunofluorescence and proteomic analyses. DdPAC stimulation in astrocytes significantly reduced GFAP expression and Aβ plaques in 5xFAD mice, while minimal effects were observed when stimulated in neurons. Conversely, neuronal DdPAC stimulation led to a significant reduction in IBA1 expression and increased it when stimulated in astrocytes. Proteomic analysis also revealed distinct responses: astrocyte stimulation showed a bigger number of differentially expressed proteins and prevalent immune activation and cytokine signalling, whereas stimulation in neurons modulated less protein numbers and involved mainly synaptic transmission. Ontology analysis, when comparing DdPAC stimulation to GFP, revealed that the variation of the proteins mainly impacts at the post-synaptic level, both when stimulated in neurons and astrocytes. The involvement of the PKA protein interactomes is greater at the neuronal level than in astrocytes of the 5xFAD mice for treatment comparison. Therefore, our data reveal distinct functions of cAMP signalling in neurons and astrocytes, while indicating that selective activation of cAMP signalling in astrocytes might be a promising therapeutic approach for AD treatment.

The perirhinal cortex is a functional hub for several memory circuits, and its activation facilitates the recuperation of multiple types of episodic memory in aging rats

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A memory is processed through specific memory circuits formed by interconnected brain areas distributed throughout the brain. Therefore, various such circuits exist in the brain, each associated with different types of memory. In this study, using a memory enhancer called RGS14414 as a gain-of-function tool, we demonstrate that the activation of a brain area within the interconnected ventral pathway circuits—primarily involved in processing information related to objects—enhances object recognition memory. In contrast, the same treatment in brain areas not connected to these circuits produced no effect, indicating which brain areas are functionally involved in this type of memory and which are not. Using this strategy for other types of memory, we found that, unlike other brain areas, the perirhinal cortex (PRh) serves as a convergence point for memory circuits associated with fear, objects, odors, spatial navigation, touch, taste, and time. This makes the PRh not only a hub for these memories but also a promising therapeutic target for treating memory deficits associated with aging. In aging, episodic memory—which encompasses recognition, spatial, and temporal components—is primarily affected in individuals experiencing memory dysfunction. Further studies in aging rats revealed that PRh activation was sufficient to both restore and prevent deficits in recognition, spatial, and temporal memories. PRh-treated aging rats exhibited complete normalization of episodic memory functions. These findings suggest that the PRh is a functional hub, and its activation could serve as a therapeutic target for combating memory deficits.

This work was supported by grants from the Ministerio de Ciencia, Innovación y Universidades (PID2022-136954OB-I00), the Junta de Andalucía (PROYEXCEL-00422), and the Fondo Europeo de Desarrollo Regional (CTS-586-G-FEDER), awarded to Z.K.

The role of cerebral vasculature and metabolism in the altered myelin pattern associated to alcohol abuse during adolescence

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Alcohol addiction often begins during adolescence, causing long-lasting neurobiological and behavioral changes that contribute to addiction. Chronic alcoholism is linked to reduced cerebral blood flow and metabolism, which are essential for myelination. Oligodendrocytes, the cells responsible for myelin production, require high energy levels supplied by blood flow. Despite evidence of alcohol-induced myelin alterations and vascular dysfunction, the connection between these processes and cognitive or behavioral impairments remains unclear.
In our study, male Wistar rats were exposed to intermittent alcohol during adolescence (3 g/kg ethanol, 4 days/week for 4 weeks). Brain tissue from the hippocampus (Hpp) and medial prefrontal cortex (mPFC) was collected at 24 hours and 2 weeks post-exposure. Immunohistochemistry with markers for myelin (MBP), oligodendrocytes (GST), and endothelial cells (Glut-1) was performed, followed by fluorescent imaging and analysis using Fiji. Histological analysis was completed with protein and RNAm expression evaluations of myelin and blood vessels proteins, as well as with magnetic resonance studies, to infer the metabolic and white matter alterations caused by alcohol consumption.
In the Hpp, alcohol increased myelinated fiber density but reduced MBP staining 24 hours post-exposure, which normalized after 2 weeks. Although vasculature density remained unchanged, Glut-1 intensity decreased, suggesting metabolic dysregulation. In the mPFC, no early changes were detected, but after 2 weeks, GST+ cell density decreased, indicating oligodendrocyte loss, while myelin patterns were preserved, suggesting compensatory overactivation of remaining cells.
In conclusion, alcohol exposure induces region- and time-specific changes in myelin and vasculature, affecting brain function. These findings highlight potential mechanisms underlying alcohol-related cognitive and behavioral impairments, opening avenues for targeted therapies to address addiction-related brain alterations.
This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project "PI22-01141" and co-funded by the European Union.

The Role of MCT8 in Brain Development and its Implications for Allan-Herndon-Dudley Syndrome

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Thyroid hormones (THs) are essential for CNS development, regulating processes such as neurogenesis, cell migration, synaptogenesis, and myelination. THs need specific transmembrane transport proteins like MCT8 to cross the cell membrane. Mutations in the SLC16A2 gene, which encodes MCT8, lead to the Allan-Herndon-Dudley syndrome (AHDS), a rare X-linked disorder that mainly affects males. MCT8 dysfunction disrupts THs transport to the brain, causing cerebral hypothyroidism and peripheral hyperthyroidism; resulting in profound intellectual disability (IQ <30), neurodevelopmental delays, and central hypotonia with spastic paraplegia, among others. Despite advances in understanding this syndrome, there is still no effective treatment for its neurological symptoms. These alterations arise during neurodevelopment, as shown in published studies from our laboratory. While the mouse is commonly used in preclinical studies, the role of MCT8 in mouse neurodevelopment remains unexplored.

This study aimed to clarify MCT8's function during murine neurodevelopment, to improve understanding of AHDS pathophysiology, and determine the optimal timing for treatment. Using immunohistochemistry, we examined MCT8 expression in wild-type mouse brains during perinatal stages. Our findings indicate that MCT8 is expressed from prenatal stages in key brain regions, including the cortex, hippocampus, and brain barriers, mirroring human patterns.

We also studied an avatar mouse model of AHDS with a point mutation in SLC16A2 (the P253L avatar mouse), using RNA sequencing, RNAscope and immunofluorescence. Our results confirm AHDS brain disorders begin prenatally, emphasizing the need for an early and effective treatment to address neurological problems early on. Additionally, as transient perinatal cerebral hyperthyroidism may affect various brain cells differently, therapies could target affected cells to reduce collateral damage. Thus, administration of TH analogues targeting specific brain cell types in the fetus is suggested as a potential therapeutic approach to alleviate the neurological problems arising during prenatal stages in AHDS.

The Role of Pcdh9 in Adult Neural Stem Cells in the Context of Glioblastoma Formation

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The subependymal zone (SEZ) in the adult brain functions as a critical neurogenic niche, playing a dual role in both neurogenesis and the pathophysiology of brain cancer. Neural stem cells (NSCs) within the SEZ are responsible for generating neurons for the olfactory bulb and can transition between quiescent, primed, and activated states. Additionally, the SEZ is thought to harbour cells that may give rise to glioblastoma multiforme (GBM), an aggressive brain tumour. Emerging evidence suggests that GBM may originate from SEZ NSCs, with glioblastoma stem cells (GSCs) sharing key characteristics with normal NSCs.

Protocadherin 9 (Pcdh9), a member of the Pcdhs family, has been identified as a potential tumour suppressor. Its downregulation is associated with a variety of cancers, including gliomas. Pcdh9 plays a pivotal role in regulating cell-cell adhesion and maintaining tissue integrity, and its loss could promote tumour growth and invasiveness in GBM. Furthermore, Pcdh9 downregulation may facilitate GBM progression by inducing epithelial-to-mesenchymal transition, which enhances tumour cell motility, invasion, and metastasis. Despite these observations, the precise mechanisms through which Pcdh9 contributes to GBM progression remain poorly defined.

In this study, we employed single-cell RNA sequencing to demonstrate that Pcdh9 is highly expressed in quiescent and primed NSCs, with reduced expression in activated NSCs and neural progenitor cells, indicating that Pcdh9 may play a role in regulating NSC dynamics and neurogenesis within the SEZ. Additionally, utilizing an innovative three-electrode configuration for SEZ electroporation, we show that downregulation of Pcdh9 alters NSC differentiation trajectories and modulates pathways commonly dysregulated in GBM.

Collectively, our findings strongly suggest that Pcdh9 is involved in the regulation of NSC dynamics, particularly in the context of GBM pathogenesis.

TOPOISOMERASES INHIBITORS AS PHARMACOLOGICAL APPROACH TO COUNTERACT TDP-43 LOSS OF SPLICING FUNCTION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease marked by motor neurons demise leading to weakness, motor incoordination and progressively to swallowing, chewing, breathing problems and death. Alteration of the protein TDP-43 is the main physiopathological hallmark of both familial and sporadic ALS cases. TDP-43 is a nuclear RNA-binding protein that regulates the alternative splicing of hundreds of exons, but in ALS-afflicted motor neurons, TDP-43 mislocates to the cytoplasm, disturbing this role. One of the main consequences is the inclusion in mature mRNAs of intronic regions that are normally excluded due to the inhibitory TDP-43 binding (i.e. “cryptic exons”, CEs). The pathological inclusion of CEs in different genes such as UNC13A or Stathmin2, normally induces a decrease in its protein levels and correlates with ALS symptoms and progression both in patients and models. Conversely, topoisomerases are enzymes dedicated to reduce DNA supercoiling during replication and transcription and its inhibitors (TI), employed normally as chemotherapies, have been recently shown to modulate alternative splicing through poorly understood mechanisms. In this study, we explored the viability of the pharmacological reduction of CE inclusion in an in vitro context of TDP-43 loss of function (LOF). We screened 1018 FDA-approved drugs in a model of TDP-43 LOF in neuroblastoma cells. From the candidate drugs unveiled, a significant number were TI, so we focused on two of them on following experiments: Aclarubicin and Topotecan. Both TIs managed to reduce the expression of the CEs analyzed in both neuroblastoma cells and iPSC-derived motor neurons. Moreover, TI seems to succeed in restoring UNC13A and STMN2 protein levels. In conclusion, TIs offer promise as a pharmacological strategy to mitigate toxic TDP-43 splicing LOF effects which define ALS. Future directions include the functional testing and in vivo essays of the Tis, as well as trying to elucidate the mechanisms implicated.

Transcranial static magnetic stimulation, a novel approach to refractory epilepsy

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Transcranial Static Magnetic Stimulation (tSMS) is a noninvasive neuromodulatory technique that has demonstrated its capacity in reducing heightened cortical excitability, a hallmark of many forms of epilepsy, a neurological disorder that affects over 50 million people worldwide, and approximately one-third of patients do not respond current pharmacological treatments. We previously demonstrated in animal models, including pharmacological, traumatic and genetic models of epilepsy, that tSMS was able to dramatically reduce the number of crisis. Hence, the aim of this project was to test the efficacy of a pilot clinical program of tSMS on refractory focal epilepsy.
The study was conducted at the University Hospitals of Coruña, Santiago de Compostela and Valladolid where six patients with refractory epilepsy received daily application of a nickel-plated NdFeB magnet (0.5 Tesla) over the epileptic focus for 30 minutes over a four-week period, guided by EEG localization. In addition, each participant had three EEG recordings: before treatment began, on the last day, and one month later.
We detected a significant decrease in the number of seizures reported during the treatment month in 5 out of 6 patients, with 2 patients experiencing complete remission of seizures during treatment. In the other 3 patients who showed positive effects, the reduction was approximately 50%, along with a decrease in seizure intensity. The patients exhibited a sustained positive effect into the following month, with a gradual return to pre-treatment values. tSMS was well tolerated, with no adverse side effects. Changes in EEG correlated with clinical signs.
Further studies with larger sample sizes are needed to confirm these initial results and explore long-term benefits. However, the findings suggest that tSMS may be a valuable addition to the treatment arsenal for refractory epilepsy.

TRANSHIPPOCAMPAL PROPAGATION OF SYNTHETIC FIBRILLAR TAU IN THE PS19 MOUSE MODEL: BEHAVIOURAL EVALUATION

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Reproducible animal models of tau propagation are required to develop new therapeutic tools for Alzheimer's disease (AD) and other tauopathies. Current murine models rely on intracranial 'seeding' of tau extracts obtained from human AD or aged transgenic mice brains, in which pathogenic tau concentration is unknown. The present study aimed to evaluate tau spread using known amounts of synthetic P301S-tau fibrils (STF, SPR-329, StressMarq) as seeds, and characterise the resulting behavioural phenotype.
Ten-week-old PS19 mice received 2 µl of STF (n=21, PS19STF, 2µg/µl) or vehicle (n=22, PS19Veh) in the dentate gyrus of the right hippocampus via stereotaxic surgery. The anxiogenic behaviour [open field test (OFT) and elevated plus maze (EPM)], cognitive performance (Barnes test) and motor coordination [rotarod test (RRT)] were evaluated 4 or 8 weeks after STF treatments. Standard immunohistochemical (IHC) analysis with AT8 and Alz-50 anti-tau antibodies were performed to address tau propagation. Statistical analysis was performed by Student's t-test and repeated measures ANOVA, followed by Tukey's test.
Four weeks after STF administration, PS19 mice did not display behavioural abnormalities compared to vehicle-treated animals. However, 8 weeks after inoculation, PS19STF animals spent less time in the correct quadrant during the Barnes test (-18±6s, $t=2.986$, $df=16$, $p<0.01$), and felt faster in the RRT (-26±9s, $t=2.954$, $df=17$, $p<0.01$) than PS19Veh mice, evidencing cognitive and motor impairments following STF treatment. IHC analyses revealed abundant neurofibrillary tangle-like accumulations in both ipsi- and contralateral hippocampi of PS19STF (but not PS19Veh) mice at both time points, indicating that STF seeds induced rapid transhippocampal tau propagation in PS19 transgenic mice.
These results support the use of STF as an experimental tool for studying tau propagation and the subsequent AD-like behavioural phenotype in PS19 mice.
Supported by grants BIO22/ALZ/002 (BIOEF/Maratoia-EiTB), IT1512/22 (Basque Government), CNS2023-143768, and FJC2022-048338-I (funded by MCIN/AEI/10.13039/501100011033 and by the European Union Next GenerationEU/PRTR)

Traumatic brain injury modulates serum extracellular vesicles subpopulations driving cerebral endothelial activation

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Extracellular vesicles (EV) are nanometric, membrane-bound structures released by all cells, playing a crucial role in intercellular communication. The amount, size and cargo of EV reflect the cellular status and vary depending on their pathophysiological context. Traumatic brain injury (TBI), acute brain lesion with an increasing prevalence and an important socioeconomic impact, is characterized by an intense neuroinflammatory response and secondary brain damage. EV have emerged as potential biomarkers of prognosis and mediators of secondary lesion in TBI. Although studies have shown that TBI increases the release of serum-derived EV in patients and animal models, cell-specific EV signalling and their effect over the neurovascular unit remains unclear.

This study aimed to identify and characterize TBI-induced EVs. EV-enriched fractions were obtained from serum of TBI patients and mice using gradient isolation and size-exclusion chromatography. EV concentration and size was determined by nanoparticle tracking analysis (NTA), while cargo was analysed by dot blot and western blot. A new protocol was developed to determine EV cell origin by detecting specific surface markers: Asgr-1 (hepatocytes), CD41 (platelets), CD14 (macrophage/microglia), IBA-1 (microglia), GFAP (astrocytes) and CD146 (endothelium). After characterization, mouse microvascular endothelial cells were exposed for 24 hours to EV from either control or TBI animals.

TBI induced a significant increase in EV derived from macrophages/microglia and astrocytes, but not from platelets or endothelial cells, suggesting an immune-inflammatory activation. TBI-EV enhanced the expression of inflammatory genes (il1b, saa3), as well as endothelial activation markers such as eNOS and VEGF, potentially contributing to blood-brain barrier breakdown. Moreover, the cargo of TBI-EV was mostly enriched in inflammatory proteins as their cargo.

In conclusion, TBI alters serum-derived EV subpopulations, increasing those that drive neuroinflammation and endothelial activation, highlighting their potential role as biomarkers and therapeutic targets.

Funding: Fondo de Investigaciones Sanitarias (FIS-ISCIII/FEDER-PI22/00362); MICIU (CNS2023-145023) and Fundación Mutua Madrileña.

Treatment with VEGF-B as a neuroprotective factor in a selective and late neuronal death´s disease

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Neurodegenerative diseases represent a great challenge and economic investment in social and health terms: the search for and development of effective treatments to counteract them has been a great challenge for science and society. Our group has extensive experience in the analysis of an animal model to develop neuroprotective strategies, the Purkinje Cell Degeneration mouse (PCD), as it has degenerative scenarios of varying severity. The neuronal death affecting the olfactory bulb (OB) of this model perfectly mimics the most prevalent human neurodegenerative processes: late and prolonged in time.
In this study we aimed at demonstrating the neuroprotective properties of a substance little known in neuroscience, the Vascular Endothelial Growth Factor Type B (VEGF-B). We wanted to know whether the systemic application of this neurotrophic factor in our model can reduce neuronal death of the OB.
To this end, we performed two different schedules of periodic intraperitoneal administration of VEGF-B, from postnatal day 40 (P40) to P90 or from P50 to P90, every five days. Both at P89 and P90, animals underwent olfactory behavioral tests, and then they were sacrificed to analyze their OB by immunofluorescence.
Our preliminary results suggest that VEGF-B increases the survival of mitral cells enhancing the number and quality of these neurons in PCD mice, which may be reflected in the olfactory capabilities of mice. Such neuroprotective role may be mediated by a reinforcement of the mitochondrial activity. In this way, we aim to take a further step towards the development of effective therapies against disorders involving massive neuronal death, especially those of the late type, as they are the most frequent in humans at an advanced age.

Turning Back Time: Harnessing Partial Reprogramming to Rejuvenate Astrocytes and Boost Brain Function

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The mammalian brain has limited regenerative capacity, leading to DNA damage accumulation, cellular stress, and functional decline with aging. This contributes to cognitive deterioration, neuroinflammation, and increased disease susceptibility. Partial Reprogramming (PR) has been shown to rejuvenate multiple tissues in mice by remodeling the epigenetic landscape, but its effects on the central nervous system (CNS) remain largely unexplored. Our research investigates the impact of PR on astrocytes, essential glial cells that maintain tissue homeostasis, support neurons, and regulate inflammation, but deteriorate with age.

We established in vitro models of aged astrocytes using two approaches: (1) replicative senescence via serial passaging and (2) long-term culture. PR was induced using a doxycycline-controlled system. Astrocyte maturation was assessed through the expression of GFAP, S100β, nestin, and AQP4, while population dynamics were analyzed by detecting Iba1 and SMA. Ki67 expression was used to examine proliferation.

To evaluate aging, we examined nuclear envelope integrity, senescence-associated β-galactosidase activity, metabolic alterations, and age-related epigenetic changes. Specifically, we assessed γH2AX, a DNA damage marker, and H3K9me3, a heterochromatin mark linked to genome stability. Aged astrocytes exhibited increased γH2AX foci and altered H3K9me3 distribution, indicating DNA damage accumulation and epigenetic drift.

Our results demonstrated that astrocytes progressively acquired maturation markers, effectively modeling astrocyte aging. Aged astrocytes displayed nuclear envelope instability and epigenetic alterations, which were significantly reversed by PR. PR restored laminB1 levels, reduced γH2AX foci, and partially restored H3K9me3 levels, suggesting enhanced genomic stability and epigenetic rejuvenation.

These findings highlight PR as a promising strategy to reverse aging hallmarks in astrocytes, paving the way for therapeutic interventions targeting astrocyte dysfunction in aging and neurodegenerative diseases.

Type I interferon pathway in Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-beta (Aβ) plaques, tau tangles, and neuroinflammation. The amyloid cascade hypothesis, which posits that Aβ accumulation initiates the disease, has dominated AD research. However, this model fails to fully explain disease progression, as Aβ plaques are also found in non-demented individuals, and Aβ-targeting therapies have yielded limited success. Genome-wide association studies (GWAS) have identified genetic loci associated with microglial signaling, directly linking inflammation to AD risk. Notably, individuals with amyloid plaques but no cognitive impairment exhibit lower inflammation levels, suggesting a protective mechanism. Here, we identify the type-I interferon (IFN-I) pathway as a central mediator of neuroinflammation in AD. Elevated IFN-I levels in wild-type mice induce key AD features, including astrogliosis, neuronal dysfunction, and axonal damage. Moreover, increasing IFN-I in an AD mouse model exacerbates pathology, while pharmacological blockade of IFN-I prevents cognitive decline. These findings highlight the pivotal role of IFN-I in AD and suggest that targeting this pathway may offer a promising therapeutic strategy.

Unveiling myeloid-derived suppressor cell activity in demyelinating lesions of Multiple Sclerosis: a comparison of traditional and AI-based methods

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Monocytic myeloid-derived suppressor cells (M-MDSCs) represent an important component of the regulatory mechanisms in immune-mediated diseases like multiple sclerosis (MS). Our group has recently described the presence of M-MDSCs associated to high inflammatory areas of MS demyelinating lesions. Interestingly, their higher density was directly associated with a less severe disease course in primary progressive MS patients. Notably, the function of M-MDSCs in MS is also a matter of debate, as circulating M-MDSCs exhibit a clear suppressive activity in relapsing-remitting MS patients, which is lost or opposite in progressive patients. Despite these results from the peripheral blood, the in situ activity assessment of M-MDSCs in MS lesions is still controversial, due to the lack of direct functional assays in human tissue. However, there are several indirect ways to infer the immunosuppressive activity of M-MDSCs, such as the evaluation of apoptosis in T cells as their canonical target cells, as well as the presence of CD84, a previously described marker of their highly immunosuppressive activity.
In the current work, we will address the morpho-functional evaluation of M-MDSC and its association with MS severity in demyelinating lesions from 17 MS patients using two different approaches: a) the conventional measurement of M-MDSC abundance by manual counting and their association to the presence of TUNEL+ CD3+T cells; b) the automated quantification of CD84 expression in M-MDSCs through the development of an AI-based neural network. Finally, we will correlate the immunosuppressive function of M-MDSCs (assessed by both methods) with the disease progression in each MS patient.
Both technical approaches for cell quantification will help us to address the activity function analysis in human tissue, shedding light into the relevance of M-MDSC role in the clinical course severity of MS.

Upregulation of voltage-gated potassium channels in peripheral blood mononuclear cells: a new approach to multiple sclerosis treatment

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Multiple sclerosis (MS) involves immune dysregulation and abnormal ion channel function. This study investigated the expression and function of voltage-gated potassium (Kv) channel isoforms (Kv1.1, Kv1.2, Kv1.3, Kv1.6, Kv4.2, Kv4.3, Kv7.2) in peripheral blood mononuclear cells (PBMCs) and lymphocytes from MS patients (remittent-recurrent) compared to controls. We found an upregulation of in six out of seven Kv isoforms in PBMCs from MS patients, with sex-specific differences observed (female showing a more pronounced upregulation of specific isoforms). Electrophysiological analysis of CD3+ T lymphocytes showed no significant differences, while CD19+ B lymphocytes displayed a notable decrease in outward current density. These findings suggest potential variations in Kv channel function across immune cell types and highlight the need for further exploration of their roles in MS pathogenesis, particularly within B lymphocytes and considering sex-based considerations.

Use of human bone marrow mesenchymal stem cell exosomes for ependymal repair and edema reduction in posthemorrhagic hydrocephalus treatment

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Germinal matrix hemorrhages and intraventricular hemorrhages (GMH/IVH) often lead to posthemorrhagic hydrocephalus (PHH), a severe cause of morbidity and mortality in premature neonates. GMH/IVH disrupts the ependyma, which acts as a functional and physical barrier between the cerebrospinal fluid and the brain tissue. As a consequence, cerebrospinal fluid circulation and physiology are severely affected; and thus, makes ependyma a chiefly important target when it comes to designing PHH treatments. Bone marrow-derived mesenchymal stem cells (MSCs) are extremely effective anti-inflammatory agents which have already shown positive results in PHH treatment. However, there is no current therapy to recover the ependyma in humans.
Human MSCs were cultured under inflammatory or non-inflammatory conditions to extract and purify their correspondent exosomal fraction through sequential centrifugation steps. The effect on the ependymal differentiation and ciliogenesis was tested in vivo and in vitro. Also, the effect on the parenchymal edema was assesed in vivo on a PHH surgical model. Conditioned and non-conditioned exosomes were used. Ventricular wall explants were analysed through confocal microscopy. Proteomic analysis and functional characterisation of exosomes was made.
Firstly, no rejection of exosomes or the human MSCs is observed. Both MSCs and exosomes have a posititve effect on ciliogenesis and edema proportion and severiy. This also indicates that exosomes are able to partially reproduce the human MSCs therapeutical effect in vitro and in vivo. Functional enrichment of proteomics gives some insight on the mechanism that makes the therapy possible.

Therefore, it is concluded that:
1.Exosomes from human MSCs emulate the cell therapeutic effect on ependymal differentiation and subventricular edema reduction
2.Functional enrichment of exosome proteomic data suggest that exosome proteins' biological functions could explain the observed therapeutic effect.

No conflicts of interest to declare.
Funding: Junta de Andalucia (UMA18-FEDERJA-277) and Instituto-de-Salud-Carlos-III (PI19/00778), Spain; co-financed by FEDER funds from the European Union.

Using hiPSC-derived neuronal models to uncover cellular and functional phenotypes associated with Sanfilippo syndrome type B

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Sanfilippo syndrome type B is a rare inherited lysosomal storage disorder caused by loss-of-function mutations in the α-N-acetylglucosaminidase (NAGLU) gene. These genetic variants lead to impaired degradation of heparan sulfate, causing its accumulation within cells and triggering neuronal damage and progressive neurodegeneration. Affected children experience severe cognitive decline, behavioral abnormalities, and premature death. Despite its devastating impact, there is currently no effective treatment, as the pathophysiological mechanisms remain poorly understood.

To investigate the alterations underlying Sanfilippo syndrome type B neuropathology, we generated 2D forebrain neuronal models and 3D cortical organoid models from control and isogenic NAGLU-mutant human induced pluripotent stem cell (hiPSC) lines. Using these models, we examined disease-associated changes at both cellular and functional levels. We utilized the genetically encoded calcium indicator GCaMP6s to perform intracellular calcium imaging throughout neuronal differentiation, enabling us to identify early disruptions in neuronal network activity and connectivity. Additionally, immunofluorescence analyses of lysosomal and neuronal markers provided further insights into the impact of NAGLU mutations on cellular integrity and lysosomal function.

Our findings reveal that mature neurons generated from NAGLU-mutant hiPSCs recapitulate key phenotypes of the disease, including early defects in neuronal activity and connectivity, as well as alterations in lysosomal dynamics. These results highlight the utility of hiPSC-based models for studying disease pathogenesis, providing insights into early functional and cellular alterations. Furthermore, these models offer a valuable platform for studying potential therapeutic strategies through high-throughput screening approaches. By deepening our understanding of Sanfilippo syndrome type B pathology, this study lays the foundation for future studies aimed at developing effective therapies for Sanfilippo Syndrome type B.

HOMEOSTATIC AND NEUROENDOCRINE SYSTEMS

Acute pesticide exposure during zebrafish development alters stress reactivity and anxiety-like behaviours

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Pesticides are widely used in agriculture, yet their long-term neurobehavioural effects. This study investigates whether acute exposure to a pesticide mixture at 0.5 µg/L, the maximum level permitted in drinking water, during early development in zebrafish (Danio rerio) results in lasting behavioural and molecular alterations. The pesticide cocktail included ziram, captan, chlorpyrifos, thiacloprid, thiophanate, boscalid, and glyphosate, reflecting environmentally relevant exposures.

Zebrafish were exposed to pesticides from 2 hours post-fertilization (hpf) to 5 days post-fertilization (dpf) and assessed at 5 dpf and 28 dpf. At 5 dpf, stress reactivity and habituation to repeated stimuli were behaviourally assessed, followed by morphological analysis and qPCR to evaluate gene expression changes. At 28 dpf, anxiety-like behaviour was examined using the Novel Tank Diving Test, followed by morphological assessment and gene expression analysis of brain samples. Molecular analyses focused on the hypothalamic-pituitary-interrenal (HPI) axis, and genes associated with oxidative stress and pesticide metabolism.

Acute pesticide exposure at 5 dpf led to increased stress-reactivity, altered habituation patterns, and morphological changes. At 28 dpf, fish previously exposed to pesticides displayed reduced anxiety-like behaviours, resembling a continuous pesticide exposure (positive control group treated with pesticides from 2 hpf to 28 dpf). However, significant HPI axis were not observed in this fish, only on those continuously exposed, suggesting different underlying mechanisms between acute and prolonged exposure.

Even brief, low-concentration pesticide exposure during early development, a window of increased vulnerability to environmental factors, can induce persistent neurobehavioral changes. These findings highlight the need for reassessment of pesticide safety thresholds. Given the relevance of zebrafish as a model for human neurodevelopmental disorders, these results raise concerns about potential long-term risks for both aquatic ecosystems and human health.

Key words: early-life exposure, neurotoxicity, oxidative stress, endocrine disruption, behavioural plasticity, environmental pollutants

Developmental, Reproductive and Metabolic effects of Endocrine Disruptors

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In everyday life people are exposed to hundreds of Endocrine Disruptors, substances that can mimic the hormone's action and interfere with the development of the human organism. My project focuses specifically on bisphenol A and bisphenol B and perfluorooctanesulfonic acid (PFOS). These substances are found in a very wide array of manufacturing such as food packaging, plastic toys, waterproofed objects. It is important to take in account that Endocrine Disruptors may produce an adverse effect when combined at concentrations at which individually it is not shown, even below the established safe threshold. Pregnant CD1 mice are treated with each individual substance or a mixture of them from embryonic day 4 to 17 and sacrificed. Samples from the mothers are taken to quantify the actual amount of accumulation. Brains of embryos are collected to understand the possible influence they can undergo indirectly. Preliminary tests seem to show more toxicity in female compared to male after an exposition of 24 hours in primary culture of atrocities treated with the mixture of the two classes. The Endocrine Disruptors considered may have a role in regulating or interfering with inflammatory or regulatory mechanisms in the environment of neurons interfering with the role of the glia.

FGFR3 in Arcuate Nucleus Astrocytes: A Key Regulator of Energy Homeostasis and Body Weight

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Obesity and metabolic disorders represent significant public health and economic challenges worldwide. The hypothalamus plays a central role in integrating metabolic signals, with the arcuate nucleus (ARC) containing key neuronal populations, including pro-opiomelanocortin (POMC) and neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons, which are essential for maintaining metabolic balance. In addition to neurons, ARC astrocytes contribute to neuronal activity and synaptic plasticity, however, their role in metabolic control remains poorly understood.

Mutations in the fibroblast growth factor receptor-3 (FGFR3) gene have profound effects on skeletal development, leading to dwarfism-associated conditions. Interestingly, those diseases are characterised also by early-onset obesity and metabolic defects. Although FGFR3 is highly expressed in hypothalamic ARC astrocytes, its implication in the central regulation of metabolism remain unclear.

In this study, we investigated an astrocyte-specific mouse model of Fgfr3 downregulation to examine its role in ARC-mediated metabolic control. We employed intrahypothalamic injections of adeno-associated viruses engineered to deliver short-hairpin RNAs (shRNAs) targeting Fgfr3, driven by the astrocyte-specific GFAP promoter.

FGFR3 downregulation in ARC astrocytes led to reduced energy expenditure and increased body weight, without changes in food intake or physical activity. Histological analysis revealed increased adipocyte size in epididymal white adipose tissue and morphological and molecular changes in brown adipose tissue. Molecular studies showed elevated glial fibrillary acidic protein (GFAP) levels in hypothalamic extracts, indicating astrocyte reactivity, but no changes in POMC or AgRP protein expression. Neurohistological assessments and in vitro studies further demonstrated astrocyte morphology and molecular signature alterations.

Our findings reveal that FGFR3 expression in hypothalamic astrocytes is essential for maintaining energy homeostasis and body weight, underscoring the importance of ARC astrocytes in central metabolic regulation and highlighting FGFR3 as a potential therapeutic target for obesity and related metabolic disorders.

Glucocorticoids regulation of VNUT: Implications for ATP and catecholamine accumulation and release

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Neurosecretion, the process by which neurons and neuroendocrine cells synthesize, store, and release signalling molecules, is essential for maintaining physiological homeostasis. ATP and other purines serve as extracellular messengers in purinergic neurotransmission, with their vesicular accumulation mediated by the vesicular nucleotide transporter (VNUT), encoded by the gene SLC17A9. Recent evidence suggests that ATP is critical for facilitating both catecholamines accumulation and release in chromaffin cells.

We hypothesize that glucocorticoids regulate VNUT expression in neuroendocrine cells, thereby modulating intravesicular levels of ATP and catecholamines and altering their exocytotic release. To test this hypothesis, we first studied the VNUT promoter to search for glucocorticoid response elements (GREs). After, PC12 cells, a chromaffin cell line, were treated with dexamethasone, to assess the impact of glucocorticoids on VNUT expression as well as on ATP and catecholamine accumulation and release.

Our study provides novel insights into the interplay between glucocorticoid signalling and purinergic as well as catecholaminergic neurotransmission. Such findings could uncover new therapeutic targets for neuronal and endocrine disorders linked to dysregulated neurotransmitter release.

Investigating the impact of Gender-Affirming Hormone Therapy on AD Pathology and epigenetic modifications in an AD mouse model

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Gender dysphoria (GD) and gender-affirming hormone therapy (GAHT) may influence Alzheimer's disease (AD) risk through epigenetic mechanisms. However, no epidemiological studies directly link GD and GAHT to AD diagnoses. This work aims to investigate the effect of GD and GAHT on AD pathology and whether epigenetic modifications associated with GD and GAHT overlap with those linked to AD risk.
To test this hypothesis, GAHT was mimicked in an AD mouse model (AppNLGF) to determine its impact on AD pathology and associated epigenetic changes. Female-to-male GAHT was induced in 12-week-old female AppNLGF mice using subcutaneous testosterone pellets, while male-to-female GAHT was induced in male AppNLGF mice using 17β-estradiol and cyproterone acetate pellets. After 80 days, cognitive and locomotion function was assessed via behavioral tests followed by blood collection and brain isolation. Our preliminary behavioral data showed no alterations in any of the groups in memory performance assessed by Y maze nor anxiety in EPM test. Interestingly, GAHT might influence locomotion and exploration only male to female AppNLGF mice, while female to male AppNLGF mice showed changes in freezing in Fear Conditioning test that could reflect hormonal effects on amygdala function, fear processing, or stress responses.
This study will provide insights into the potential impact of GAHT on AD risk as identifying shared epigenetic modifications between GD, GAHT, and AD pathology. These findings may improve our understanding of hormone therapy's role in neurodegenerative disease progression.

Mild traumatic brain injury provokes microglial priming in the hypothalamus, enhanced neuroinflammation and altered behavior after acute psychological stress

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Microglia are activated by neuroinflammatory insults, and this activation is usually tightly controlled and transient in duration. Following traumatic brain injury (TBI), however, microglia may become “primed”, hence displaying exacerbated inflammatory responses to secondary stimuli, what augments cognitive and behavioral deficits. Psychological stressors may enhance the reactivity of primed microglia. The objective of this study was to determine to what extent mild TBI-induced microglial priming results in enhanced neuroinflammatory and neuroendocrine responses to the Acute Social Defeat (ASD) model of psychological stress. Adult male mice were subjected to mild TBI by a midline fluid percussion. Fourteen days later, control and TBI mice were exposed to a 2-hour cycle of ASD. Then, hypothalamic neuroinflammation and HPA axis activation were assessed, along with animal behavior. Morphological analysis of microglia located in hypothalamic paraventricular nucleus (PVN) revealed decreased microglial size and complexity (therefore increased activation) in mice that received both TBI and ASD. These mice also displayed exacerbated neuroinflammatory activation in the hypothalamus, e.g. increased expression of key cytokines and chemokines (IL1B, IL6, CXCL1 and CCL2), protein receptors (TLR4) and inflammatory pathways (NFKB1). Besides, the expression of behaviorally relevant neurohormones (AVP and OXT) in the hypothalamus was also increased, and CRH neuropeptide did accumulate in PVN (indicating enhanced feedback inhibition mediated by corticosterone of the HPA axis in TBI x ASD mice). Finally, ASD provoked behavioral alterations, such as anxiety-like behaviors in the open field test or aversion to new objects in the social avoidance test. Overall, these data provide evidence of primed microglia in the hypothalamus after diffuse TBI, resulting in an amplified neuroinflammatory response and altered behavior to an acute stressor. Thus, mild TBI represents a risk factor that may disturb the response to future episodes of acute stress.

Sex and Age-Specific Alterations in Oxytocinergic Neuronal Networks

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Oxytocin is a crucial neuropeptide primarily synthesized in hypothalamic nuclei, where it regulates parental behaviors, social interactions, and pro-social neuromodulation, playing a vital role in establishing social bonds. The proper development of oxytocinergic circuits supports these functions, influencing sex-specific and context-dependent social behaviors critical for survival and adaptation to changing environments. However, the structural and functional changes in these circuits during key life stages, such as motherhood or aging, remain insufficiently explored.

To address this, we employed the iDISCO+ tissue clearing method combined with light-sheet fluorescence microscopy to generate a three-dimensional reconstruction of the oxytocinergic system in adult and aged mouse brains. Our analysis revealed an increase in the number of oxytocin-positive (OXT+) cells in the supraoptic nucleus (SON) and in the anterodorsal preoptic nucleus (ADPN) of postpartum females, whereas no significant changes were observed in other major oxytocinergic regions, such as the paraventricular nucleus (PVN). In aged mice, we detected a reduction in OXT+ cells within the bed nucleus of the stria terminalis (BNST) of females and the ADPN of males. Furthermore, these nuclei exhibited a decreased proportion of magnocellular OXT+ cells, a distinct oxytocinergic neuronal subtype. Our findings suggest that further modifications may occur under pathological aging conditions.

These results provide new insights into the plasticity and dynamic, sex- and region-specific changes in oxytocinergic circuits throughout development, motherhood, and aging.

Thyroid Hormone Deficiency Disrupts Oxytocin Synthesis and Induces Structural Remodelling in the Hypothalamus

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Thyroid hormones play a crucial role in numerous physiological processes and are essential for proper brain development and function. In adults, thyroid dysfunction has been associated with emotional disorders such as anxiety and depression. However, the mechanisms by which thyroid dysfunction contributes to these disorders remain poorly understood. Oxytocin (OXT), a neurohormone synthesised in the hypothalamus, acts as a neurotransmitter regulating social behaviour and emotional responses. Interestingly, in the 1990s, a connection between thyroid hormones and OXT was established, revealing that thyroid hormones regulate the transcription of the OXT-encoding gene. Despite the relevance of these findings, this relationship has received little attention in recent decades.

In this study, we investigated the impact of thyroid hormone deficiency on OXT synthesis in hypothyroid mouse models using immunolabelling and transcriptomic approaches. We found that hypothyroidism significantly reduces OXT synthesis in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. Notably, arginine vasopressin synthesis, which also occurs in magnocellular neurons of the PVN and SON, remains unaffected by hypothyroidism. Furthermore, thyroid hormone deficiency induces structural remodelling in the SON and PVN, including astrocytic retraction and synaptic changes in OXT-producing neurons, which may contribute to the observed reduction in OXT synthesis. Transcriptomic analysis of the PVN in hypothyroid conditions suggests additional molecular pathways involved in thyroid hormone-dependent regulation of OXT synthesis. These findings provide new insights into the relationship between thyroid hormones and OXT, highlighting the potential role of reduced OXT levels in mediating the adverse emotional effects associated with thyroid dysfunction. Future studies may uncover novel perspectives on the pathophysiological mechanisms underlying anxiety and depression.

CIRCUIT DYNAMICS AND OSCILLATIONS

Biofilms as Bioelectric Networks: Understanding Their Response to Neural Signals

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Information processing in biological systems presupposes communication, whether with other organisms, the environment (learning and stigmergy), or one's past or future selves (memory and prediction). How do individual cells (neurons, bacteria) use communication to build emergent complex beings like brains and colonial super-organisms? How does information within a whole, such as a vertebrate animal or biofilm, relate to the information processed by its individual components (cells)? Given that both neurons and bacteria utilize bioelectrical signals, could this serve as a mechanism for intercellular communication between these two entities?
Our previous studies demonstrated that bacteria respond specifically to neurotransmitter cues. We analyzed Escherichia coli, Limosilactobacillus reuteri, and Enterococcus faecalis, key members of the human microbiota, with the latter two species capable of transitioning to pathogenic states. Neural-type stimuli altered bacterial membrane potential (Vmem) without affecting growth or viability, indicating that bacteria can depolarize or hyperpolarize in response to external signals. However, whether biofilms—structured bacterial communities with enhanced information processing capabilities—exhibit dynamic responses to neural signals remains unknown.
In this study, we investigated the bioelectrical responses of bacterial biofilms to neural stimuli. Using the Vmem-sensitive fluorescent dye thioflavin T, which indicates hyperpolarization, we monitored 24-hour-old biofilms over three hours under confocal microscopy, comparing untreated biofilms to those exposed to glutamate (Glu). Our results reveal that bacterial biofilms exhibit a coordinated bioelectrical response to Glu exposure, distinct from planktonic cultures. Unlike individual bacterial cells, which respond independently, biofilms displayed a synchronized shift in Vmem upon exposure to neurotransmitters, suggesting intercellular communication within the biofilm structure.
These findings suggest that biofilms, much like neural networks, utilize dynamic and synchronized bioelectrical signals to process external stimuli. This opens new avenues for understanding bacterial behavior and targeting bioelectric signaling at the interface of bacteria and neurons, particularly in systems such as the microbiota-Gut-brain axis.

Cooperative action of interneuron classes supports the hippocampal spatial code

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Identifying the computational roles of different types of neurons is a prerequisite for understanding neural networks. Most of this cortical neural diversity is embodied in various types of GABAergic interneurons, grouped into four major families: Pvalb, Sst, Vip, and Id2 neurons. We have collected an extensive dataset of opto-tagged neurons from all four families, along with Camk2-expressing excitatory neurons, from the neocortex and hippocampus. The physiological features of the opto-tagged neurons were used to train a machine learning classifier, which was subsequently employed to accurately infer specific interneuron types in large-scale recording experiments. By combining targeted optogenetic manipulations with neuron type classification, we reconstructed the circuit's synaptic connectome. The simultaneous activity of interneurons predicted the animal's position to the same extent as excitatory neurons, with distinct contributions from different types. Using both functional connectivity analyses and targeted optogenetic manipulations, we demonstrate that interneuron types differentially control the magnitude of spiking within the place field and contribute uniquely to the different features of place fields of pyramidal neurons. Our findings assign a prominent role of interneurons to the flexible cognitive map.

Endocannabinoids signaling regulates sharp-wave ripples function across learning

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The hippocampus is crucial for learning and memory. Within this structure, inhibitory interneurons play a fundamental role in shaping network activity by regulating neuronal excitability and synchronizing oscillatory patterns, such as sharp-wave ripples (SPW-Rs), which are strongly linked to memory. Endocannabinoids (eCBs), acting primarily through type-1 cannabinoid receptors, have been shown to be involved memory and neuronal activity. However, their precise role in hippocampal network dynamics, particularly in relation to SPW-Rs and memory function, remains poorly understood. Therefore, to elucidate the role of eCBs in hippocampal network dynamics, we chronically implanted high-density silicon probes coupled with optic fibers in CA1 to simultaneous record extracellular recordings and endocannabinoid dynamics using the GRABeCb2.0 sensor expressed in all hippocampal neurons (n = 6 mice). This approach enabled the measurement of the temporal relationship between eCBs release and hippocampal dynamics, including SPW-Rs. Mice were exposed to a rewarded spatial alternation task across 10 days. Data showed a strong correlation between eCBs fluctuations and SPW-Rs events which were modulated across learning. These results suggest a fundamental role of eCBs in shaping memory consolidation and provide a novel framework for studying eCB-related cognitive impairments.

GIRK channels regulate CA2 oscillatory activity and social memory in early Alzheimer's disease

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Social memory, the ability to distinguish familiar from novel individuals, is impaired in the early stages of Alzheimer's disease (AD), when amyloid-β oligomers (oAβ) are still soluble. The hippocampal CA2 region plays a pivotal role in the formation and integration of this type of memory, although it remains poorly studied. Here we hypothesized that under physiological conditions, CA2 oscillatory activity underlies social memory and that modulation of GIRK channels, essential for controlling neural excitability and shown capable of rescuing CA1-dependent oscillatory activity and memory in early AD, could also benefit CA2 network activity and restore social memory impaired by oAβ.

To test this hypothesis, we examined CA2 oscillatory activity while animals performed social memory tests in a model of early amyloidosis, induced by intracerebroventricular injections of $oA\beta_{1\text{-}42}$ in both sexes. Furthermore, we assessed whether these deficits could be reversed by selective pharmacological activation of neuronal GIRK channels.

Our results revealed a specific pattern of oscillatory activation in naïve mice associated with sociability and social memory with sex differences, whereas $oA\beta_{1\text{-}42}$ impaired this type of memory and disrupted CA2 oscillatory activity. Moreover, selective GIRK activation restored sociability, social memory and oscillatory activity, suggesting a previously unrecognized role of GIRK channels in CA2-dependent alterations observed in amyloidosis models.

In conclusion, we characterized CA2 network activity during social memory formation and retrieval, identifying sex dimorphism in oscillatory dynamics in naïve animals, alterations induced by $oA\beta_{1\text{-}42}$, and their reversal through pharmacological GIRK channel activation in an AD-like amyloidosis mouse model. This study advances our understanding of the neural basis of social memory in both health and disease and establishes GIRK channels as key regulators of CA2 network dynamics and social memory processes in the early stages of AD

MCIN/AEI/10.13039/501100011033 (PID2020-115823-GBI00), JCCM/ERDF (SBPLY/21/180501/000150), UCLM/ERDF (2022-GRIN-34354) to JDNL and LJD. ANDALUSIA/ERDF (UPO-1380660), MCIN/AEI (PID2022-141997NB-I00) to RSC.

Inhibitory determinants of memory consolidation

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Replay of neuronal activity patterns during hippocampal sharp-wave ripples (SPW-Rs) is necessary for learning. Several lines of evidence suggest that synaptic plasticity involving local interneurons is fundamental for SPW-Rs function: 1) pyramidal neurons experience four times more inhibition than excitation during SPW-Rs; 2) SPW-Rs inhibition biases neural sequences; and 3) SPW-Rs are locally generated events in a brain region lacking excitatory-to-excitatory connections. Still, how synaptic plasticity involving local interneurons controls memory consolidation remains unclear. Here, we investigated the inhibitory mechanisms underlying hippocampal reactivation. First, we used high-resolution optogenetics and large-scale recordings in behaving mice to synthetically coactivate (6 pulses at 100 Hz, every 0.2-1 seconds) pairs of CA1 pyramidal neurons. The increase of cofiring during SPW-Rs after the protocol strongly correlated with synthetic co-activation strength ($\rho < 10^{-24}$). Moreover, while a fraction of pairs increased their SPW-Rs co-firing (4.73%), a similar fraction of pairs (4.28%) decreased it, suggesting inhibitory mechanisms. Next, we investigated these plasticity motifs in behaving mice during natural learning. Neural activity patterns forged during maze exploration were reactivated in the subsequent SPW-R events, showing both positive and negative coactivation signatures between CA1 pyramidal neurons, resembling the plasticity dynamics observed after synthetic coactivation. Furthermore, high-resolution optogenetics probing revealed an increase in the inhibitory input to pyramidal neurons during SPW-Rs, which positively correlated with the spatial information content of the neurons. These results support a direct role of synaptic plasticity from local interneurons in memory reactivation. To causally test this hypothesis, we chemogenetically perturbed local CA1 interneurons using excitatory DREADDs and measured changes in the pyramidal neurons' pairwise coactivation. While the average population activity during SPW-Rs remained unchanged, a reorganization in the neural population code drastically disrupted memory reactivation. Our findings demonstrate how synaptic inhibition from interneurons is necessary for memory.

Integrating Social Signals into Spatial Maps: Hippocampal processing of conspecific cues during exploration

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Mice rely on olfactory and vomeronasal marks to recognise other individuals, which need to be integrated into the spatial map to encode the identity of other conspecifics in a context-dependent manner. Our objective is to explore the influence of vomeronasal information in the spatial map characterising the functional interconnection between the chemosensory amygdala and the hippocampus.
We assessed vomeronasal influence on spatial mapping using two different behavioural paradigms: (1) a head-fixed virtual maze where female mice explore visually and olfactorily cued sectors and (2) an Enriched Open Field (EOF) test designed to provide ethologically relevant spatial cues. Neural activity was recorded using electrophysiological implants in the hippocampus and/or posteromedial cortical amygdala, allowing us to assess how vomeronasal inputs shape spatial representations.
The neuronal activity associated with the exploration of the labyrinth showed specific responses to given sectors. These responses include both increased and decreased firing rate usually in the sector where male urine is delivered. In the EOF, in contrast to our expectations, female mice avoid the area where the male left marks. But regarding the neuronal activity, we could identify neurons whose response is associated with male-marked areas.
Our findings suggest that vomeronasal cues significantly affect spatial representation in the hippocampus, likely via amygdalo-entorhinal pathways These experiments broaden our knowledge about how neuronal activity links both exploration patterns and specific locations in space in the context of the integration of social cues.

Local cortical desynchronization in layer 5 under general anaesthesia: wakefulness-like activity without consciousness

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Consciousness, defined as the ability to perceive and react to one's surroundings, is typically suspended during both sleep and general anaesthesia. Traditionally considered a global brain state, consciousness is now understood as a dynamic interplay between widespread and localized neural processes. Local sleep has been observed during wakefulness, and local wakefulness during sleep, but how these dynamics operate under general anaesthesia remains unclear. Recent studies suggest that loss of consciousness is linked to widespread cortical synchrony, particularly in layer 5 pyramidal neurons across sensory, motor, and associative regions. If this synchrony is essential for anaesthetic-induced unconsciousness, inducing local wakefulness in layer 5 should not be possible under deep anaesthesia—an assumption that warrants further testing. To explore this, we used chemogenetics to selectively activate layer 5 pyramidal neurons in the sensorimotor cortex of mice under isoflurane anaesthesia. This intervention caused a localized shift from slow-wave activity to tonic firing, resembling wakefulness at a local level. Notably, this disruption reduced synchrony between homologous layer 5 regions in opposite hemispheres but did not alter the overall depth of unconsciousness or the transitions into and out of anaesthesia. These findings suggest that while widespread layer 5 synchrony may support anaesthetic unconsciousness, it is not strictly required under isoflurane anaesthesia. Our results demonstrate that local wakefulness-like activity can occur in deep anaesthesia without restoring consciousness, highlighting the need for further research into the balance between local and global neural states in anaesthesia-induced unconsciousness.

Local modulation of cortical emergent activity by temperature

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The mammalian neocortex exhibits recurrent and rhythmic activity during physiological slow-wave sleep and under anesthesia. This global activity is highly synchronous and is characterized by the slow oscillation rhythm (SO), which alternates between periods of high neuronal activity (Up state) and silence (Down state) at a frequency of 0.3-1 Hz. While asynchronous activity is expected in the awake and healthy brain, SO dynamics have been locally observed around brain lesions (e.g., traumatic brain injury or stroke) in awake patients [1]. Previous work demonstrated that raising temperature from 34°C to 40°C enhances cortical synchronization [2]. Furthermore, it has been measured that brain lesions lead to localized temperature increases around the damaged area [3]. Here we aimed to determine whether brain temperature increases following lesion contribute to perilesional SO wave activity.

To this end, we studied the impact of local increases in temperature in an in vitro preparation of desynchronized cerebral cortex slices, while recording with a multielectrode array. For this investigation we used a novel probe that can locally increase the temperature in four locations. For monitoring, we used a high-resolution thermographic camera capable of spatiotemporal monitoring with 0.1°C precision. This set up allows us to concomitantly monitor and modulate temperature both temporally and spatially. Under these experimental conditions, we have observed that local temperature elevations enhance SO synchronization and increase the likelihood of a cortical region becoming the source of slow waves. This effect diminishes as the distance from the heated region increases. These findings suggest that local changes in temperature could be a contributing factor to slow wave generation in perilesional areas.

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Pre- and postsynaptic determinants of claustro-cortical connectivity

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The claustrum (CLA) is a thin and elongated brain structure located between the insula and lateral striatum, implicated in a wide range of behaviors. It is characterized by its extensive synaptic connectivity with multiple cortical regions. While CLA projection neurons are glutamatergic, several studies have shown an inhibitory impact of CLA on its cortical targets, suggesting the involvement of inhibitory cortical interneurons. We employed in vivo and ex vivo electrophysiology and optogenetics in mice to dissect the synaptic organization of projections from CLA to the anterior cingulate cortex (ACC), a robust claustro-cortical pathway implied in cognitive functions. Optogenetic stimulation of CLA neurons expressing CaMKIIa or VGLUT2 evoked distinct multiphasic excitatory and inhibitory responses in the ACC that depended on the stimulated CLA population and the recipient layer in the ACC. Stimulation of CLA-ACC axons evoked monosynaptic responses in pyramidal neurons and four types of molecularly defined interneurons. Synaptic responses were uniquely determined by the presynaptic CLA projection type, the recipient cortical layer, and the type of postsynaptic neuron, with interneurons displaying the highest specificity. This intricate organization of the CLA-ACC pathway explains the diverse and complex influence of CLA on cortical activity.

Prefrontal-hippocampal neural dynamics as useful biomarkers of cognitive impairment and rescue in schizophrenia: Role of gamma oscillations and serotonin receptors

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The Cognition, Neural Networks and Neurotechnology laboratory (www.puiglab.org) investigates dysfunctional neural networks and circuits underlying intellectual disability in preclinical models of schizophrenia. We focus on prefrontal and hippocampal neural activities, paying special attention to the circuit's connectivity. Recent studies by the group unravelled a functional disconnection between the medial prefrontal cortex and the dorsal hippocampus during psychosis and memory impairment associated with NMDAR hypofunction (Delgado-Sallent C et al., 2022, 2023), a pathophysiological mechanism relevant for schizophrenia. More specifically, during psychosis induced by the NMDAR antagonist phencyclidine, aberrant gamma (~60 Hz) and high frequency (~160 Hz) bands emerged within prefrontal microcircuits whereas overall activity in the dorsal hippocampus decreased. We are currently mapping the emergence of these abnormal neural networks within the distinct areas of the medial prefrontal cortex and investigating the underlying cellular mechanisms via chemogenetics. The prefrontal-hippocampal disconnection also correlated with short- and long-term memory impairment assessed by the novel object recognition test following subchronic administration of phencyclidine. Together, our results suggest that dysfunctional prefrontal-hippocampal communication plays critical roles in cognitive impairment observed in schizophrenia. Another main aim of the laboratory is to find novel therapeutical targets for cognitive amelioration produced by new generation antipsychotic drugs. Interestingly, among highly prescribed antipsychotic medication, the ones showing the most pro-cognitive abilities bind strongly to one or more serotonin receptors. To gain further insight into the distinct roles of serotonin receptors in cognition and intellectual disability, we have investigated at a cellular and functional levels the contribution of serotonin 5-HT1A, 5-HT2A, 5-HT4 and 5-HT7 receptors to the modulation of prefrontal-hippocampal circuits and to the pro-cognitive actions of several antipsychotic compounds, including risperidone (5-HT2AR) and lurasidone (5-HT7R).

Role of GIRK channels in social decision-making, social memory and oscillatory activity of the reward circuit

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Decision-making between social and food rewards is a key survival process, coordinated by neural circuits that integrate memory with emotional and motivational information. Dysfunction within these networks may be involved in neuropsychiatric disorders where social interaction is impaired. GIRK channels, which are essential for regulating neuronal excitability, are critical memory modulators, although their specific role in these circuits have been still poorly understood.
In this study, we have investigated the effects of the administration of GIRK modulators, a specific opener and a selective blocker, directly into the prelimbic cortex (PrL), a region critically involved in decision-making processes. To do so, we used a social versus food preference paradigm with simultaneous electrophysiological recordings from four key regions of the social-motivational circuit: CA2 hippocampal subfield, PrL, basolateral amygdala (BLA), and nucleus accumbens (NAc).
Our results showed that the pharmacological modulation of GIRK channels activity did not reverse the preference for food rewards over social stimulus. Interestingly, while repeated exposure to the same conspecific leads to a progressive decrease in social interaction attempts with both vehicle (control) and neuron-GIRK activator treatment, we found a significant increase in the number of attempts to access the social door with the selective GIRK blocker treatment. This suggests that loss of GIRK function in the PrL may interfere with social memory processes, affecting the recognition of familiar individuals. Furthermore, these behavioral alterations are associated with impaired brain oscillations in the areas under study and disrupted connectivity between them.
Our findings provide new insights into the role of GIRK channels in social decision-making and open the door to new potential therapeutic targets for disorders characterized by social memory deficits and altered social behavior.

MCIN/AEI/10.13039/501100011033 (PID2020-115823-GBI00), JCCM/ERDF (SBPLY/21/180501/000150) and UCLM/ERDF (2022-GRIN-34354) to JDNL and LJD.
MCIN/AEI/10.13039/501100011033/FEDER/EU (PID2021-122446NB-I00) to AG and JMD-G.

Study of sequential dynamics in central pattern generator circuits through perturbation of electrically coupled neurons

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In central pattern generator cricuits, dynamical invariants have been found recently in the form of cycle-by-cycle relationships that build robust neural sequences. In motor circuits, it is common to build the phases of the rhythm from mutual inhibition of the neurons involved, and frequently electrical synapses are also found.In this study, we analyze the effect of different closed-loop perturbations on electrically coupled neurons that are part of a central pattern generator to determine the associated modulation of the variability of intervals in the sequence, synchronization and dynamical invariants. For this purpose, we introduced three different perturbations into the PD neurons of the pyloric CPG: (i) A Hindmarsh-Rose (HR) model neuron electrically coupled to a PD neuron through an artificial gap-junction, thus implementing a biohybrid circuit with a dynamical-clamp protocol; (ii) A square pulse current injection delivered at the depolarization phase of each PD burst; (iii) An artificial electrical synapse that aims to cancel out the natural electrical coupling between the two PD neurons.
Additionally, we recorded extracellularly from the LP neuron to quantify the LP-PD delay, an interval that builds a dynamical invariant with the cycle-by-cycle period. All three perturbations affected the rhythm variability and the electrical coupling. The biohybrid coupling with an artificial Hindmarsh-Rose also affected the shape of the invariant changing the intercept of the linear relationship. All protocols have been proven effective to study the relationship of electrical coupling and sequential dynamics with the help of real-time closed-loop neurotechnologies.

Understanding human brain function: light-controlled communication in human brain connectoids

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The human brain is an extraordinarily complex organ composed of neurons and glial cells that operate across specialized regions to regulate cognitive and motor functions. Neurodegenerative diseases disrupt these intricate networks, yet traditional models, including animal systems and 2D cell cultures, fail to fully replicate key human brain characteristics. Brain organoids (BOs), three-dimensional cultures derived from human stem cells, provide a more advanced alternative by mimicking the structural and molecular features of brain development.

Current assembloid technology enables unguided connectivity between brain regions represented in BOs. Our study aims to model neurodegenerative diseases by establishing guided connectivity to BOs, preventing the assembloids physical fusion of BOs, and maintaining the anatomical isolation of distinct brain regions while allowing guided axonal connection.

We generated patterned BOs of brain regions relevant to neurological disorders using human iPSC cells. These BOs represent dorsal forebrain cortex and striatum and incorporate the optogenetic tools channelrhodopsin and halorhodopsin for light control of both depolarization and hyperpolarization of neurons, as confirmed by calcium and chloride imaging assays.

Using a custom-designed microfluidic device, we have developed connectoids - regionally patterned human BOs interconnected by axonal bundles. Our results demonstrate that mature BOs successfully form connections growing 3 to 9 mm long axonal bundles. Neuronal activity can be controlled by channelrhodopsin and halorhodopsin activation, inducing either robust neuronal firing response or hyperpolarization, respectively.

By establishing an interconnected BO model with tunable neural activity, we provide a novel platform for investigating mechanisms of neurological diseases and conducting pharmacological studies in a human context.

Unraveling inhibitory connections in vivo using large-scale extracellular recordings and high-resolution optogenetics

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Hippocampal neuronal dynamics are fundamental for episodic memory formation and consolidation. Inhibition is expected to play a key role in organizing memory representations by regulating these neuronal dynamics. Resolving inhibitory connections in extracellular recordings in vivo is challenging because they often cause changes in the membrane potentials of low firing rate neurons, including pyramidal cells, without impacting significantly their rate. We present a method to unravel inhibitory synaptic connections using low-density, high-resolution optogenetics in the CA1 region of the hippocampus. We reasoned that neurons are less excitable when being synaptically inhibited by interneurons. The decrease of neuron's excitability to be experimentally activated when the stimulation coincides with an interneuron's activity reveals an inhibitory connection. We first validated our approach using neural network simulations and whole-cell paired recordings in vitro. Then, we scaled up our method using large-scale recordings and high-resolution optogenetics in the hippocampus of freely-moving mice. We examined all possible interneuron-pyramidal pairs and developed a multi-step approach to identify putative inhibitory pairs: 1) Classification of light-responsive pyramidal cells; 2) Identification of interneuron-pyramidal pairs exhibiting a significant decreases of light-evoked responses when stimulation coincidences with the spikes of the putative presynaptic interneurons (bootstrap, $p<0.001$, 500 repetitions), 3) Selection based on effect size, considering only pairs with a reduction in light responses exceeding 5 Hz. Preliminary analysis suggests a connection probability between inhibitory and pyramidal cells of 0.17 (0.01+/-0.02). Using our validated inhibitory pairs in vivo; we developed a classifier based on features of the unstimulated pairwise cross-correlogram of the neurons, enabling the identification of inhibitory connections across our full dataset (> 15,000 cells, 24 mice). This work establishes a novel approach to unravel inhibitory connections in vivo using large-scale single units recording and high-resolution optogenetics. Future studies will integrate simultaneous identification of excitatory/inhibitory connections exploring synaptic dynamics during learning.

NEW METHODS AND TECHNOLOGIES

Effects of Transcranial Static Magnetic Stimulation on Lower Limb Corticospinal Excitability: A Preliminary Study

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Neurophysiological techniques that can induce plasticity, modulate cortical excitability or produce interference with normal brain activity are known as neuromodulation techniques. The main objective of this proposal is to describe and characterise the effects that the application of transcranial static magnetic field stimulation (tSMS) applied over the vertex for 30 minutes on the lower limbs of healthy subjects.
We performed 18 experimental sessions in 9 healthy subjects (6 females and 3 males; mean age 38.0 ± 12.9; range 25-56). Each subject participated in two stimulation sessions, one real tSMS and one sham tSMS over the location of cZ during 30', in a randomized order, at least six days apart.
We evaluated corticospinal excitability before and after the tSMS application with transcranial magnetic stimulation. We acquired the Resting motor threshold (RMT) of the relaxed abductor hallucis (AbdH). We elicited 5 motor evoked potentials (MEPs) with a stimulus intensity of 120% of RMT and 5 at 150% of RMT, recording the electromyographic activity of the relaxed AbdH, tibialis anterior (TA) and rectus femoris (RF) muscles of both legs.
A significant difference was found between the MEP elicit with the intensity of 120% of RMT in the interaction between the time of the evaluation (pre or post stimulation) and the treatment (real or placebo) in the TA and in the AbdH.
This will be a preliminary study to evaluate the clinical potential of this new non-invasive neuromodulation technique in a target that may be very important in pathologies such as spinal cord injury, amyotrophic lateral sclerosis and Parkinson's disease, the lower limbs.

A state-of-the-art method for high-throughput, automated locomotor activity testing in neuropharmacology, safety, and toxicology studies

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Quantifying locomotor activity, stereotyped movements, and anxiety-related behaviors is crucial in neuropharmacology, safety, and neurotoxicity studies to assess psychostimulant, sedative, or toxic effects of new compounds. Conducting large-scale locomotor assessments under GLP compliance is labor-intensive. To address this, we developed the VivaMARS system, enabling automated, high-throughput locomotor activity testing for up to 30 rodents per session. This study validates VivaMARS using two reference compounds, caffeine (CAF) and chlorpromazine (CPZ), known to increase and decrease activity, respectively. Adult Sprague Dawley® rats (6-7 weeks, 250-300 g) and CD1 mice (4-5 weeks, 25-35 g) were divided into five groups (N=5 rats, N=3 mice per group): saline, low-dose, and high-dose for each compound. CAF (4 mg/kg, 16 mg/kg) and CPZ (3 mg/kg, 10 mg/kg) were administered intraperitoneally (IP). Data was recorded for 60 min post-CAF and 30 min post-CPZ. Parameters measured included total activity, divided into activity with displacement (ActiD) and without displacement (ActiND), distance traveled, vertical activity, immobility time, and speed. Data acquisition and analysis were performed using GLP-compliant Ponemah software. CAF-treated groups showed increased activity across all parameters except immobility time, which decreased. CPZ had the opposite effect. Our findings demonstrate that VivaMARS is a powerful and efficient platform for acute locomotor activity testing in rodents, with further validation needed.

Abnormal hyperactivity of specific striatal ensembles encodes distinct dyskinetic behaviors revealed by high-resolution clustering

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L-DOPA-induced dyskinesia (LID) is a debilitating complication of dopamine replacement therapy in Parkinson´s disease and the most common hyperkinetic disorder of basal ganglia origin. Abnormal activity of striatal D1 and D2 spiny projection neurons (SPNs) is critical for LID, yet the link between SPN activity patterns and specific dyskinetic movements remains unknown. To explore this, we developed a novel method for clustering movements based on high-resolution motion sensors and video recordings. In a mouse model of LID, this method identified two main dyskinesia types and pathological rotations, all absent during normal behavior. Using single-cell resolution imaging, we found that specific sets of both D1 and D2-SPNs were abnormally active during these pathological movements. Under baseline conditions, the same SPN sets were active during behaviors sharing physical features with LID movements. These findings indicate that ensembles of behavior-encoding D1- and D2-SPNs form new combinations of hyperactive neurons mediating specific dyskinetic movements.

Advancing Glioma Treatment by Evaluating Direct Light Therapy in Neuronal Networks

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Glioma, an aggressive brain tumor with limited treatment options, has been increasingly targeted with light-based therapies like photodynamic therapy (PDT). PDT uses photosensitizers to trigger oxidative stress, to which cancer cells are particularly sensitive. Part of this occurs through singlet oxygen ($^1O_2$), which is more direct and potent than other reactive oxygen species. Within the EIC-funded GlioLight Project, we are collaborating to develop and validate an innovative Direct Light Therapy for glioma. This approach utilizes deep-penetrating 1267 nm light to directly generate $^1O_2$ without requiring photosensitizers. Our group aims to characterize the efficacy of this therapy both in vitro and in vivo, as well as its safety.

We assessed the therapeutic potential of 1267 nm light by exposing mouse astrocytoma cell lines (GL261 and SMA-560), which will also be used for the in vivo studies, to laser doses ranging from 450 J/cm² to 1400 J/cm², and assessing their effects on cell viability, cell death, singlet oxygen ($^1O_2$) production, and mitochondrial superoxide formation. To investigate safety, we first established and characterized a mouse primary neuronal-astrocytic cortical culture to model neuron-glia interactions and synaptic transmission, verifying its reliability in representing healthy brain conditions in terms of cell composition and network activity. Subsequently, we exposed these cells to the same laser doses and post-treatment assays as above, but additionally measured the effects on neuronal networks by performing network and population dynamics analysis of calcium imaging recordings, using our in-house-developed Netcal software.

By bypassing photosensitizers, this novel laser approach may offer a safer and more efficient alternative for glioma treatment, paving the way for non-invasive, targeted light-based therapies.

Aloe vera in solution and in blended nanofiber scaffolds: A study on neurite outgrowth potential

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This study investigated the potential of commercial aloe vera (AV) to promote neurite outgrowth in an in vitro model of acute axotomy, using organotypic dorsal root ganglia (DRG) explants (n = 230) derived from neonatal rats (n = 15). Neurite outgrowth was evaluated based on two criteria: the percentage of viable explants—defined as having neurite outgrowth exceeding 1.5 times the explant diameter—and the longest neurite length. Explants were cultured under control conditions, with AV-supplemented media (AV-dilution), and on nanofiber scaffolds composed of poly-L-lactate (PLLA), polydioxanone (PDS), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), and blended PHBV/AV. After six days in culture, explants were immunostained with the neuron-specific marker Tuj1 and the Schwann cell marker S100. Neurite measurements were obtained using ImageJ software, and data were analyzed using the statistical program Jamovi.

Both the control and AV-dilution groups promoted radial neurite outgrowth, whereas all nanofiber scaffolds induced bidirectional neurite growth along the fiber orientation. Binomial logistic regression revealed that viable explants were significantly more likely in the control group compared to PDS ($p = 0.0042$) and PHBV ($p < 0.0001$). Additionally, AV-dilution significantly increased viability compared to PLLA ($p = 0.0459$). The blended PHBV/AV scaffold demonstrated higher viability odds than PLLA ($p = 0.0479$), PDS ($p = 0.0001$), and PHBV ($p < 0.0001$). Among groups with comparable viability odds (control, AV-dilution, and PHBV/AV), the longest neurite lengths were also similar, as confirmed by the Kruskal-Wallis test with post hoc pairwise comparisons (n = 120).

In conclusion, the control, AV-dilution, and PHBV/AV conditions yielded the highest likelihood of viable explants and the longest neurite outgrowth. This study is the first to demonstrate the direct permissive effects of AV on axonal outgrowth. Furthermore, the PHBV/AV scaffold showed considerable potential for controlled axonal regrowth and Schwann cell migration, underscoring its promise as a biomaterial for tissue engineering applications.

An innovative approach for conducting 3D electrophysiological recordings within intact organoids

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Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 headstage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth, and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

Bioprinting of Human Dental Pulp Stem Cells using collagen-fibronectin bioink for autologous personalized 3D neurogenic models

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The development of patient-specific technologies is essential for advancing neural tissue engineering. These technologies are not only crucial for cell therapy but also for studying disease mechanisms and tissue development. Bioprinting human stem cell-derived tissues offers a method to create specialized structures that closely mimic native tissue architecture and function. 3D bioprinted tissues provide enhanced structural and physiological accuracy for in vitro models, serving as platforms to investigate cell-to-cell and cell-matrix interactions, refine drug testing, and improve our understanding of neurodegenerative disease progression.
Bioprinting can be used with somatic cell lines as well as different types of stem cells, from ESCs to MSCs and iPSCs. However, the generation of personalized 3D models requires patient-derived cells to reflect accurately individual characteristics of neural tissue. Despite patient-derived neural stem cells or iPSCs being promising alternatives, they present medical challenges, such as the limited availability and the difficulty to obtain, or the risks of genetic instability, respectively.
Human dental pulp stem cells (hDPSCs) are derived from the neural crest representing a promising alternative to iPSCs for personalized therapies and disease modeling. Their recently demonstrated ability to generate neuronal action potentials along with their resistance to hypoxic environment makes them suitable for bioprinting patient-specific 3D neural tissues.
We assessed in this work the viability and neural differentiation of bioprinted hDPSCs in collagen-fibronectin bioink at different concentrations (7×10^5, 1.4 and 2.4×10^6 cell/scaffold) after a 14-day neural differentiation protocol. Live/dead assays confirmed cell viability, while the downregulation of the mesenchymal marker Vimentin, and the expression of neuronal differentiation markers—early (DCX, TUJ1) and mature (NeuN, MAP2)—along with GFAP and S100b for glial phenotypes, indicated successful neural differentiation.
In conclusion, we demonstrate the feasibility of bioprinting of hDPSC-derived neurogenic cells, validating the use of hDPSCs for 3D neural tissue modeling.

CAN WE IMPLEMENT VIRTUAL CONTROL GROUPS TO ENHANCE ETHICAL AND EFFICIENT NEUROSCIENCE RESEARCH?

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The VICT3R project (www.vict3r.eu) aims to create a comprehensive database of historical control data to reduce the use of concurrent control groups through the development and implementation of Virtual Control Groups (VCGs). By minimizing animal use and aligning with the 3Rs principles, VICT3R seeks to enhance ethical standards while simultaneously improving research consistency and reliability. The project addresses key challenges, including data collection, curation, AI-driven VCG generation, and validation in compliance with Good Laboratory Practice. While its primary focus is on repeated-dose toxicity studies, VICT3R's tools are also being applied to other study types, particularly CNS safety pharmacology, which shares fundamental endpoints with academic behavioural neuroscience research.

Further integrating academic research presents specific challenges, such as variability in study designs, test protocols, and information gaps, which must be addressed to ensure robust VCG integration. To tackle this, we are implementing an exhaustive protocol taxonomy to improve report accuracy, consistency (i.e., controlled terminology), and cross-study comparability within a database that compiles data from diverse sources. Using Natural Language Processing tools, we extract key parameters from published studies—including study design, experimental conditions, tests, and results—to build a comprehensive database and analyse variations in common behavioural tests (e.g., open field, elevated plus maze). This information will be curated by our team in collaboration with the original researchers, enhancing research report quality.

This database will support the generation of VCGs and facilitate protocol consultation and optimization by providing a repository of well-documented experimental designs. Beyond its immediate applications, the database will enable retrospective studies, in silico predictive modeling, a deeper understanding of biological variability, increased statistical power, and improved reproducibility—all advancing meta-research without requiring additional animal use.

We invite researchers to join this transformative initiative, fostering more ethical research practices while enhancing scientific outcomes.

Comparative Analysis of EEG Acquisition Systems: Neuroscan with EasyCap vs. OpenBCI with Florida Research Cap

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Electroencephalography (EEG) is essential in cognitive neuroscience, traditionally using wet electrode systems like Neuroscan for high signal fidelity and broad scalp coverage. Recently, dry electrode systems, such as OpenBCI, have gained attention for their faster setup and increased participant comfort. This study directly compares these two systems in both resting-state and Go/NoGo tasks.

Twenty participants (Mage = 20.1, SD = 2.07) completed two EEG sessions on the same day, first using OpenBCI (16 channels) and then Neuroscan (61 channels). Neuroscan data were downsampled to 125 Hz, and analyses were restricted to the matching 16 channels. Resting-state EEG included 3 minutes with eyes open and 7 minutes with eyes closed. The Go/NoGo task consisted of 200 trials (80% Go, 20% NoGo) to assess inhibitory control. EEG data were bandpass-filtered (0.5–45 Hz), and Independent Component Analysis removed artifacts before extracting ERP components (e.g., N2, P3) and performing time-frequency analyses.

Neuroscan demonstrated higher signal-to-noise ratios, lower impedance, and more robust ERP components, notably a stronger N2 in the Go/NoGo task, with fewer rejected epochs. OpenBCI enabled faster setup (<10 min) and was rated as more comfortable. Although more susceptible to motion artifacts, OpenBCI's topographic and temporal EEG characteristics were comparable to Neuroscan. Resting-state time-frequency analyses also showed similar results across systems.

Neuroscan provides superior signal quality for applications requiring high precision, such as source analysis and connectivity studies, but demands longer setup and specialized gel application. OpenBCI, while slightly noisier, offers a quick, user-friendly alternative suitable for portable EEG, mobile BCI, and large-scale studies. Future research should refine dry electrode technology, improve artifact suppression, and expand channel counts to further bridge the gap between dry and wet EEG systems.

Controlling maturation variability in human brain organoids derived from different human iPSCs cell lines

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Human brain organoids, generated from induced pluripotent stem cells (iPSCs), are valuable models for studying brain development and neurological disorders. However, a key challenge remains the variability in maturation rates and cellular composition among organoids derived from different iPSC lines, which can hinder reproducibility and comparability across studies.

Efforts to standardize organoid maturation have focused on refining differentiation protocols, optimizing culture conditions, and incorporating bioengineering strategies, as extensively documented in the scientific literature. While single-cell RNA sequencing represents the gold standard for assessing organoid maturation, its high cost and technical complexity place it beyond the reach of many laboratories.

In this study, we analyzed the expression of a selected set of genes and proteins that could serve as practical markers for assessing brain organoid maturation in standard laboratory settings. We will present data obtained from RNA sequencing, TMT-based proteomics, RT-PCR, and Western blot analyses to establish a set of reference genes and their corresponding proteins. This standardized approach aims to facilitate reliable comparisons of brain organoids derived from different human iPSC lines, ultimately improving reproducibility and robustness in the field.

We specifically focus on the overexpression of cell-type-specific markers that emerge during brain organoid maturation, such as astrocyte-specific gene expression, and the regulation of post-translational modifications of neuronal markers. This approach provides a clearer view of the organoid's stage of maturity and contributes to the establishment of reliable molecular benchmarks for assessing brain organoid development and further comparition between brain organoinds from different patients and genetic background.

Acknowledgements
We sincerely thank the Proteomic Unit and the Microscopy Unit at the Instituto de Salud Carlos III (ISCIII) for their technical support and expertise. We also acknowledge the financial support from the Ministerio de Educación, Ciencia y Universidades PID2020-115592RB-I00 to E Cano and PID2021-128087OB-I00 TO MJ Barrero.

Design of a novel blood-to-brain immunotherapy targeting Galectin-3 in primary and secondary brain tumours

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Primary and secondary brain tumours are treated by surgery, radiation therapy or chemotherapy. Unfortunately, current treatments also create an immunosuppressive scenario that may facilitate tumour growth and survival. Additionally, the tumour microenvironment is strongly immunosuppressed, further enhancing this pro-tumoural context. Therefore, based on our previous works, we have designed a novel immunotherapy targeting Galectin-3, a major driver of immunosuppression. This lectin protein is highly expressed in tumour-associated microglia, supporting the anti-tumour immune response within the brain. To increase efficiency, we have designed a strategy to allow our construct to cross the blood-brain barrier (BBB) and, thus, further analyse the effect into the tumour microenvironment.
The construct is based on the well-tested Gal-3 inhibitor: GB1107. We added an effective brain-crossing peptide, e.g. Angiopep-2 (AP2), connected by a linker, resulting in the synthesis of our novel GB-AP2 construct. This GB-AP2 could also be tagged by fluorescent molecules (e.g. 6-FAM) , that would help us to track our molecule in vitro and in vivo. We have tested the pharmacokinetics (e.g. structural integrity, binding and crossing properties) by mass spectrometry, in vitro permeability assays and immunofluorescence staining. An in vitro BBB model using mouse and human brain blood vessel cell lines was created to further evaluate these features. Finally, we analyse its anti-tumoural effects in vivo, using syngeneic mouse glioblastoma and breast cancer brain metastasis models, delving into the inflammatory state of the tumour microenvironment, particularly focused in TAMs.
Our results are:
* We successfully synthetized GB-AP2
* GB-AP2 enhance GB1107 crossing properties in vitro
* GB-AP2 modulates the TME enhancing inflammation
In conclusion, GB-AP2 may arise as a novel concomitant therapy for glioblastoma and breast cancer brain metastasis

Engineering Neuronal Identity Using Synthetic Gene Cascades

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Neuronal loss due to injury or neurodegenerative diseases such as Parkinson's leads to irreversible damage and persistent functional impairments in the human brain. This is largely because the adult brain has minimal neurogenic capacity, making natural neuron regeneration highly limited. Cell therapy has emerged as the most promising strategy for neuronal replacement in adults.

A particularly innovative approach is in vivo reprogramming, which leverages existing mature brain cells to generate new neurons. This technique relies on the overexpression of specific transcription factors to redirect cell identity toward desired neuronal types. However, traditional methods often fail to accurately replicate the intricate developmental processes governing neuronal differentiation, reducing the fidelity of reprogrammed cells to native neurons.

Here we present an advanced in vivo reprogramming strategy designed to mimic the natural gene expression sequences that drive neuronal differentiation. Our method combines CRISPR activation technology with two inducible recombinases to achieve precise, sequential activation and deactivation of key transcription factors, enhancing both the efficiency and fidelity of neuronal production. This approach holds great potential for improving neuron replacement therapies, offering a more precise and effective solution for restoring brain function.

Enhancement of human dental pulp stem cells differentiation with retinoic acid and potassium chloride generates electrophysiological activity showing neuron-like cells

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Human dental pulp stem cells (hDPSCs) represent a promising alternative for central nervous system (CNS) cell therapy due to their neural crest embryonic origin, their expression of neural markers and their ability to secrete neuroprotective factors. However, the possibility to generate functional neurons with electrophysiological properties remains a subject of debate, necessitating the refinement of ex vivo differentiation protocols. In this study, we optimized neurodifferentiation protocols for hDPSCs to enhance their neuronal potential.

hDPSCs were expanded either in the presence or absence of serum, followed by cell differentiation for 21 or 60 days using Neurocult neurodifferentiation media, either with or without retinoic acid (RA 3 mg/ml) and 40 mM potassium clorhide (KCl) pulses. Afterwards, differentiated hDPSC were characterized by immunocytochemistry, quantitative Real-Time PCR, morphometric analysis and electrophysiology. Furthermore, hDPSCs labelled with GFP were injected in non-immunocompromised C57BL6/J mice and their survival and dispersion were characterized one month post-graft.

Neurodifferentiation resulted in a significant decrease in the expression of stem cell markers. Furthermore, the expression of GFAP, S100β and p75NTR was influenced by the presence or absence of serum during the initial cell expansion phase. Notably, hDPSCs previously grown as serum-free floating spheres exhibited in vitro expression of neuronal markers such as doublecortin (DCX), neuronal nuclear antigen (NeuN), Ankyrin-G and MAP2. Presynaptic vGLUT2, Synapsin-I, and excitatory glutamatergic and inhibitory GABAergic postsynaptic scaffold proteins and receptor subunits were also present in these neurodifferentiated hDPSCs. KCl and RA treatment increased the transcripts levels of voltage-dependent Na+ and K+ channels. Consistently, these cells also displayed TEA-sensitive voltage-dependent K+ and TTX-sensitive Na+ currents as well as spontaneous electrophysiological activity and repetitive neuronal action potentials with full baseline potential recovery.

Our study highlight the importance of choosing an adequate protocol to differentiate hDPSCs, demonstrating their capacity to differentiate into neuronal-like cells that display functional excitability.

Evaluation and intervention using virtual reality for alcohol and cannabis use in youth. Psychophysiological insights

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Adolescence and young adulthood are crucial stages in life, marked by physical, emotional, and cognitive changes. However, alcohol and cannabis use can interfere with this development and have a significant measurable impact. Virtual Reality (VR) has recently been introduced as an innovative tool for both evaluation and intervention of these drug-induced alterations.
Here we present, on the one hand (1): our research on the VR-based assessment of the psychophysiology of stress-related alterations in young alcohol and cannabis users and, on the other hand (2): a recent VR-based intervention program to reduce drug consumption in this population.
In the evaluation study (1), we designed a virtual elevated plus-maze (VR-EPM) for analyzing the stress-related responses of behavior, psychophysiology (electrocardiogram and electrodermal activity), and hormones (salivary cortisol and alpha-amylase) in young people (mean age: 19.2±0.10) with problematic alcohol use (AU, n=27), alcohol combined with cannabis consumption (AU+C, n=10) and in a control group (CO, n=33). The AU group made fewer entries into the open arms compared to the CO group, while both experimental groups spent less time at the end of the open arms and had a lower time-by-look-down index than the CO group. The AU+C group displayed a higher electrodermal activity compared to the CO group. Concerning hormonal responses, the alpha-amylase to cortisol ratio, a biomarker of stress dysregulation, was higher in the AU group.
In the VR-based intervention program (2), the standards of randomized controlled trials (RCT) will be followed for young people (13-21 years old) with alcohol and cannabis use, selected from various centers managed by GINSO association in collaboration with Innovae for the VR software development. The RCT protocol with the VR-based sessions for intervention with the psychophysiological measures are presented in this communication. The effectiveness of VR for the evaluation and intervention of this population is discussed.

Exploring Nanoparticle-Based Therapies for Spinal Cord Injury

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Spinal cord injuries (SCIs) cause irreversible neuronal damage, chronic inflammation, and fibrosis, limiting recovery. The Piezo4Spine Project develops biomaterials with therapeutic molecules to promote neural repair, specifically targeting Piezo1, crucial for neuronal survival, differentiation, and inflammation.

We have developed iron oxide nanoparticles (IONPs) coated with various biocompatible polymers, thereafter functionalized with therapeutic molecules targeting Piezo1 and inflammation and tested in vitro with embryonic neural progenitor cells (ENPCs) and NR8383 macrophages. First, the viability and differentiation degree of ENPC cultures exposed to IONPs were characterized at different maturation stages (7, 14, and 21 days) using confocal microscopy and flow cytometry to assess specific neural markers and Piezo1. Additionally, we have analysed their mechanical properties using Atomic Force Microscopy (AFM) to further understand the implication of Piezo1 in neural cell responses. Force-distance curves have been obtained on the neuronal soma, providing insights into how cellular stiffness changes during neural differentiation in vitro and after exposure to IONPs. The dose with a higher biocompatibility response was selected for further studies and exposure to magnetic stimulation under an alternating magnetic field (AMF).

The polymer-coated IONPs developed have been successfully functionalized with molecules targeting Piezo1 and anti-inflammatory molecules and examined in ENPCs and NR8383 macrophage cultures in vitro, respectively. Results demonstrate high viability and a significant impact in cell differentiation in both cell types. Early attempts to incorporate these therapeutic IONPs in hydrogel matrices have been also initiated and promising results in terms of biocompatibility have been already obtained. These therapeutic platforms will be further translated into in vivo models in rats once their optimization is complete.

Altogether, our findings establish the groundwork for a novel nanomedicine-based approach in SCI repair targeting both Piezo1 and inflammation, paving the way for future in vivo applications.

Flow Cytometry-based model to study neural stem cell division asymmetry

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Somatic stem cells transit between an active and a quiescent state. Regulation of this transition is crucial to ensure the production of progeny, as well as the long-term maintenance of the stem cell pool. It has been reported that neural stem cells (NSCs) from the subependymal zone (SEZ) mainly divide in a symmetric pattern, producing either two NSCs (duplicative division) or two transitory amplifying progenitors (consuming division). Nevertheless, it has been suggested that the two NSCs derived from a duplicative division may not be identical, with one remaining as a proliferative NSC while the other returns to a quiescent state. However, studying such dynamics can be challenging. Here, we have developed a flow cytometry-based system that allows the discrimination between symmetric and asymmetric cell division events by quantifying the presence of specific markers in each of the resulting cells after a recent mitosis. It is based on (1) the fact that NSCs, when cultured in floating conditions, remain attached after mitosis, allowing just one round of division to form a 'cell pair' or doublet, (2) very diluted seeding conditions that prevent fusion of events, making possible the identification of cells generated after a single division within the first 24 hours after seeding, and (3) the differential display of the height and width parameters of the fluorescence of specific markers acquired from such doublet events in comparison to individual cells. To test the model, we have used BMP-4, a well-established pro-quiescence signal in the field, and EGFR expression, a marker of NSC activation, to investigate whether it can induce an asymmetric "return to quiescence" post-division. This new method enables the study of how different mechanisms regulate the mode of division of NSCs in vitro.

Functional imaging, optical manipulation and connectivity of biological and artificial neurons

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Recording of the membrane voltage of neurons with fluorescent indicators is one of the most promising tools to answer big questions in neuroscience, with potential of moving from highly specialised labs of indicator developers to a broader research community. Integrating it with optogenetics opens the possibility to all-optical electrophysiology to both detect and manipulate the neural activity of single neurons and their populations.

The project further explores the interface between biological and artificial neural networks through all-optical methods. We aim to establish and characterize communication between in vitro neuronal cultures and hardware-implemented spiking neural networks. Our approach combines microscopy imaging of genetically encoded calcium and voltage indicators with patterned optogenetic stimulation at single-cell resolution.

Our experimental model utilizes primary rat cortical neurons, which are well-characterized in terms of their in vitro development and activity. We employ one-photon wide-field calcium imaging and a Digital Micromirror Device stimulator to project precise patterns of light for photostimulation at cellular resolution. Through viral transduction using recombinant adeno-associated viruses, we express both channelrhodopsin and the red calcium indicator. This setup enables us to build libraries of spontaneous and evoked dynamics that serve as maps for optogenetic stimulation. We use novel genetically encoded voltage indicators from the ASAP family, which offer superior trafficking and photophysical properties developed by St Pierre lab.

This neurotechnological project bridges several disciplines and involves strong collaborations to analyze spontaneous and evoked neural dynamics and infer neuronal connectivity, and develop artificial neural networks embedded on hardware boards and create pipelines for real-time signal extraction, enabling both one-way and two-way interactions.

This combination of functional calcium or voltage imaging and optogenetics serve as a technological platform for investigating excitation-inhibition balance in neural circuits, modeling diseases in vitro, and potentially correcting aberrant activity patterns—such as those observed in epilepsy—through hub neurons.

Generation of novel BK channel-based calcium sensors using the circularly permuted HaloTag

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In neurons, Ca2+ sources and Ca2+ sensors are located 20-50 nm apart, in subcellular “Ca2+ nanodomains”. This tight coupling is crucial for the functional properties of synapses and neuronal excitability. One key player in many types of neuronal nanodomains is the large conductance Ca2+ and voltage-gated K+ channel (BK), which is characterized by synergistic activation by Ca2+ and membrane depolarization. BK potassium channels, due to their location within the cell and their function in Ca2+ nanodomains, are excellent candidates for detecting and signaling local changes in [Ca2+] occurring in specific neuronal nanodomains. A line of work in our laboratory consists of generating fluorescent variants of the channel that report BK activity induced by Ca2+ binding1. In this work, we aim to create and deploy a novel generation of these BK fluorescent constructs, using a recent strategy that incorporates the self-labeling enzyme circularly permuted HaloTag (cpHalo Tag) into specific sites of the Ca2+-dependent machinery2. The preliminary results we have obtained so far indicate that the BK964cpHalo is a very promising candidate for generating novel optoelectrical reporters.

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Human-Based Models for Neurotoxicity: Advancing Neurodevelopmental Research Beyond Animal Testing

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Neurodevelopment involves complex processes such as neuronal differentiation, migration, and synapse formation. Disruptions in these early stages are linked to neurodevelopmental disorders. Human pluripotent stem cells (hPSCs) offer a powerful tool for generating diverse neuronal populations and modeling critical developmental processes. However, further refinement is required to capture cellular interactions and maturation more accurately.

Here we present novel hPSC-based in vitro models to evaluate the neurotoxic effects of several substances during human brain neurodevelopment. We differentiate hPSCs into ventral forebrain neurons, allowing toxicity assessment at key stages such as neuroepithelium and telencephalic progenitor formation. This model also enables the evaluation of the effects of toxicant exposure on mature neurons. To enhance high-throughput screening, viability assays are conducted in 96-well plate cultures.

To better capture brain complexity, we have developed a system that allows us to analyze neuronal connectivity. This microfluidic platform integrates striatal, cortical, and dopaminergic neurons, mimicking the basal ganglia circuitry. The brain-on-chip provides insights into synaptic plasticity and neuronal communication, assessing network formation, connectivity, and functional responses to toxicants. Notably, results indicate improved functionality in mature striatal neurons upon receiving cortical inputs.

All these models leverage the human relevance of advanced non-animal methods, offering a more comprehensive approach to studying how toxicants disrupt early brain development. The new models enable the identification of early-stage alterations in viability, proliferation, and neuronal function, advancing neurodevelopmental research beyond traditional methods.

In search of biomarkers of TDP-43 alterations in the blood of patients with TDP-43 proteinopathies

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Given the necessity to identify a biomarker for the early diagnosis of ALS and other TDP-43 proteinopathies that can be easily integrated into clinical practice, we explore the measurement of alterations in TDP-43 protein in peripheral blood cells, including its expression, relative nuclear/cytoplasmic location, and its splicing functionality, as potential early biomarkers for TDP-43 alterations in these disorders.

We conducted a prospective case-control study and obtained blood from recruited patients diagnosed with ALS, or FTD (behavioral variant), and sex-age matched healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated using EDTA tubes and Ficoll gradient separation, and serum samples were also collected. Patients were checked for the most commons mutations. We explored and optimized three promising approaches for detecting TDP-43 alterations in blood: a) screening and analysis of antibodies for quantification of total and isoform-specific TDP-43 levels in PBMCs by western blot, b) assessment of nuclear-cytoplasmic translocation in PBMCs by confocal imaging, and c) detection of functional alterations, mainly changes in splicing regulation by TDP-43, through targeted RNA analysis.

Preliminary results indicate an increased tendency for TDP-43 translocation to the cytoplasm versus controls, functional alterations, and differential expression of TDP-43 isoforms in ALS patients compared to age-matched healthy controls. Those changes were not similarly observed in FTD patients.

Early detection of alterations in TDP-43 proteinopathies using accessible tissues, such as blood, would represent a significant advancement in the field of neurodegeneration. Furthermore, this project would contribute to a deeper understanding of the pathological mechanisms of TDP-43 beyond the neuronal systems.

In vivo photopharmacological neuroinhibition of brain hyperactivity involved in focal seizures with PhotoTheraPorts

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Technological advances in chemistry and biophysics have enabled the development of light-based therapies, which are emerging as targeted treatments for various medical conditions. In this project, PHOTOTHERAPORT, we combine two innovative nanotechnologies that involve light-emitting “ports” (polymer embedded with nanocrystals) and photoactivable neuroinhibitors (derivatives of the antiepileptic drug carbamazepine activated by UV light).
Proof-of-concept experiments are being conducted in various preclinical models of epilepsy. Epilepsy is a disorder characterized by recurrent seizures due to excessive neuronal excitability, affecting patients' daily lives, with 30% remaining resistant to current treatments.
Our goal is two-fold: first, to ensure the safety and biocompatibility of the implants in the brain and second, to evaluate the effectiveness of PhotoTheraPort-mediated modulation in reducing seizure activity in vivo. Pilot experiments are currently assessing any neuroinflammation (Iba-1), scar generation (GFAP), and cell death (Cas-3) caused by the ports when implanted chronically both at epicortical and intracortical configurations. Moreover, in anesthetized mice, we administer either convulsant drugs alone or in combination with photoswitchable neuroinhibitors that have been pre-illuminated or not. Preliminary findings indicate that ultraviolet preactivation of the drug reduces epileptic activity, highlighting its potential efficacy.
The Proof-of-Concept demonstrations validate the use of light-based therapies for the photopharmacological modulation of focal epileptic seizures, with initial assays and immunohistochemical analysis of PhotoTheraPorts yielding promising results. PHOTOTHERAPORT aims to develop innovative biophotonic technologies for the safe and localized delivery of light to the central nervous system, addressing a critical gap in current treatments.

Mueller Matrix-based polarimetry as a potential tool for neuroanatomy and neurosurgery

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Mueller polarimetry is a relatively novel tool for tissue study based on the inspection of polarimetric observables arising from the analysis of the Mueller matrix (MM). The MM relates the Stokes vector describing an incident polarized light beam with the resultant Stokes vector after the interaction of polarized light with a probe tissue, changing the polarimetric properties of the initial light. Polarimetric-related optical parameters as diattenuation, azimuth, indices of polarimetric purity and retardance can be derived from the MM. Among them, the indices of polarimetric purity measure the degree of disorganization of a totally polarized light beam after tissue interaction, and azimuth measures changes in the angle of oscillation of the incident polarized light. Therefore, both properties are strongly related to the internal organization of the tissue and, particularly azimuth, has been used to identify regional differences in the directionality of brain white matter fibers. In this work, filtered indices of polarimetric purity are used to study white matter tracts in post-mortem mammalian brains cut in coronal sections, allowing discrimination between 6b layer, subcortical white matter, thalamocortical radiations, optical radiations, tapetum, and fascicles within the corpus callosum. Also, subtle changes in the indices of polarimetric purity values are observed within the thalamocortical projections, suggesting an ability to identify small fascicles. Different cortical areas also show different polarimetric properties. Possible implications of this non-destructive and non-invasive technique for white matter tract analysis and tractography are discussed

Nanopatterned bioresorbable elastomeric scaffolds to promote neural, glial and endothelial differentiation using human embryonic and induced pluripotent stem cells

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Neurodegenerative disorders represent some of the most formidable medical challenges nowadays. Recent progress in neural tissue engineering has demonstrated that biocompatible materials hold significant potential for regenerating damaged neural tissues, offering promising improvements to current therapeutic approaches. Bioresorbable nanopatterned scaffolds functionalized with polydopamine (PDA) and graphene oxide (GO), have been shown to promote the differentiation of murine neural stem cells (mNSCs) towards neural and glial lineages. Herein, our aim is in one hand, to evaluate the utility of these functionalized biomaterials for the in vitro culture and differentiation as a basic research knowledge of human embryonic stem cells (hESCs), and in the other hands to test the use of human induced pluripotent stem cells (hiPSCs) as a future platform for future cell therapy. Our results show that PDA and GO scaffolds allow the in vitro culture of hESCs and hiPSCs, guiding their alignment and migration processes and maintaining pluripotency characteristics. Upon differentiation culture media, PDA and GO nanostructures direct the specification of cells towards neuroectoderm germ layer and neural crest, enhancing their differentiation capacity into neural and supportive glial cells from central nervous system (CNS) and Schwann cells from peripheral nervous system (PNS). Moreover, the functionalization with PDA and GO also support the differentiation of hESCs and hiPSCs towards endothelial precursors. Overall, these findings represent a novel in vitro culture platform to trigger the differentiation of human pluripotent stem cells to neural and vascular cells with potentially relevant uses in personalized medicine and regenerative cell therapy.

Nanoporous Membranes for Controlled Intrathecal Delivery of Advanced Therapies to the Central Nervous System: In-Vitro Study for Proof-of-Principle

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The delivery of advanced therapies to the central nervous system is challenging due to physiological barriers, low drug concentration at target sites, and systemic toxicity. Intrathecal administration offers a promising solution by bypassing the blood-brain barrier, enabling higher drug concentrations at specific brain regions while minimizing systemic side effects. Nanoporous membranes integrated into intrathecal devices have been proposed as an innovative approach to enable continuous release of advanced therapies carriers. However, ensuring efficient delivery without compromising the integrity of drug carriers remains critical. This proof-of-principle study evaluates the in vitro performance of nanoporous membranes for controlled drug delivery, focusing on the release rate and the integrity of liposome-based nanocarriers.

An in vitro transwell system was employed to assess the release profile of fluorescent liposomes (PC:CHO:DSPE-PEG2000, Rhod 560/583; 100 nm in diameter) through polycarbonate membranes with varying pore sizes (100, 200, 400 and 600 nm) at 37ºC and 5% CO_2. The release rate was determined by measuring fluorescent intensity in the receiving chamber at 24, 48 and 72 hours. The integrity of the nanocarriers was assessed by analyzing particle size, morphology and concentration, using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and electron microscopy (both SEM and TEM).

Preliminary results indicated that membrane pore size influenced the release rate, with larger pores generally resulting in higher release rates. The nanocarriers generally maintained their spherical morphology and structural integrity after passing through the membranes under the tested conditions. However, a greater degree of deformation was observed when using more restrictive membranes (i.e., 100 nm). Further in vitro studies are required to evaluate nanocarriers release in a continuous flow system, as well as the biocompatibility and functionality of membranes in cell culture models, before progressing to a functional intrathecal prototype and subsequent in vivo validation.

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Funding: MICIU, AEI (CPP2022-009810; PTQ2022-012644)

Open-access freely usable neural networks for the analysis of neuronal death in the injured spinal cord

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Cellular death, particularly neuronal death, is a central event in spinal cord injury (SCI) pathophysiology and a major determinant of the resulting functional deficits. Histological images are a basic source of spatial and temporal information on neuronal death in animal models of SCI. Recent developments in our capability of managing and analysing large quantities of data have opened the opportunity to reconstruct the cellular organization of the spinal cord before and its changes following injury. In a previous study, we compared manual, threshold, and neural network-based tools to identify and spatially locate neurons in images of full spine sections. Our analyses demonstrated the outperformance of a commercial neural network in terms of efficacy and efficiency. Here we aim to develop this approach by generating improved free open neural networks to segment neurons and anatomical structures in histological sections. Building upon the state of the art, we have used a 2D Attention U-Net trained with a dataset of more than 300 immunofluorescence images from naïve and injured mice thoracic spinal cords stained with neuronal marker NeuN and nucleus marker DAPI. The resulting deep learning networks are capable of 1) identifying and segmenting neurons in sections from both naïve and injured spinal cords; and 2) differentiating gray and white matters, the ependimum, as well as foldings and artifacts. Both networks together constitute a fast and effective tool for the identification, segmentation and spatial positioning of neurons in anatomical context and can be employed in follow-up processes such as image registration and 3D reconstruction. They are freely available at BioImage Model Zoo through the user-friendly BiaPy –an open source library and application that streamlines the use of common deep-learning workflows for bioimage analysis tasks– and its detailed tutorials.

Optogenetic modulation of cardiac function in freely moving mice

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Mental and cardiac health are intricately linked, as exemplified by a number of clinical studies showing increased risk of heart disease in patients suffering from depression or anxiety-related disorders. The autonomic nervous system links brain and heart, serving as a crucial network element for bidirectional information flow. Yet, little is known about the circuit mechanisms underlying heart-brain communication and how cardiac feedback to the brain, a process termed interoception, influences affective behavioral states.

Cardiac optogenetics allows light-mediated excitation of the heart with high spatiotemporal resolution and in a cell-type specific manner, providing an ideal method to investigate heart-brain interactions. Here, we present a methodological approach to modulate heart rate in freely moving mice by optogenetic activation of cardiac-innervating neurons. Systemic injection of a viral vector with tropism for the peripheral nervous system in a Cre-transgenic mouse line allowed for specific expression of the highly sensitive red-light activated opsin ChRmine in cardiac cholinergic neurons. To illuminate the right atrium, a region of the heart where most of the postganglionic parasympathetic neurons are located, we used an implantable µLED device that integrates a flexible magnetic loop antenna for wireless power delivery. Optical stimulation of ChRmine-expressing cardiac cells in freely moving mice resulted in a decreased heart rate.

While still in preliminary stages, our work opens up new possibilities for optogenetic modulation of cardiac function in vivo, a stepping-stone to study the causal influence of heart dynamics and interoceptive processes on brain function and behavior.

Reconstructing a 3D blood-brain barrier using pluripotent stem cell differentiation

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Brain disorders have a high societal and economic burden, affecting about one in three Europeans. Among them, neurodegenerative diseases are characterized by the progressive loss of neurons and other brain cells, which leads to cognitive and motor impairments. While current treatments can slow disease progression or alleviate symptoms, they fail to stop neurodegeneration, highlighting the need for regenerative approaches.
The 4-DBR project aims to develop a 3D bioprinting strategy to create a vascularized mini-brain that can be transplanted into patients affected by neurodegenerative diseases. To achieve this, we have established new protocols to differentiate distinct cell types from human pluripotent stem cells (hPSCs).
First, we have set up a protocol to generate striatal projection neurons (SPNs), a primary target of neurodegeneration in Huntington's disease. Our results show that hPSCs acquire SPN features when differentiated in 2D and 3D on a brain extracellular matrix hydrogel, expressing the neuronal markers MAP2, GABA, and CTIP2. Additionally, we have progressed in deriving endothelial cells and pericytes, key components of the blood-brain barrier (BBB). We demonstrate that endothelial cells expressing CD31 and von Willebrand factor can be obtained from hPSCs after 7-10 days of differentiation. The combination of different cell types with hPSC-derived endothelial cells, both in 2D and 3D cultures, generates cells expressing the pericyte marker PDGFRβ and induces the formation of tubular structures that resemble blood vessels.
In conclusion, the protocols presented here progress towards replicating a 3D BBB, laying the foundation for a bioprinted, vascularized mini-brain designed for transplantation in patients with neurodegenerative diseases such as Huntington's disease.

Relationship between power, wavelength, delivery mode, and effect in infrared-laser neural stimulation

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In the search of novel neurotechnologies for localized non-invasive neural stimulation, infrared laser stands as a promising technique for research and clinical applications. In a recent work it was demonstrated that continuous-wave near-infrared laser illumination effectively modulates neural dynamics in both continuous and activity-dependent operation modes. Despite the growing body of research in this field, several questions remain unanswered regarding the biophysical mechanisms involved in the modulatory effect (ionic channels, capacitance...) and the source of that effect (photo-thermal, photo-chemical...). Studying in detail how power, distinct laser wavelengths and illumination modes affect the neural system can help set the basis for future research steps and applications.

In this work, we analyze the data obtained by combining intracellular recordings (with sharp electrodes) and laser stimulation focused on specific neurons (using a collimator and lenses to maximize the power density and focus). We explored the illumination at distinct power and laser wavelengths when applying multiple laser illumination configurations in two different systems and stimulation modes (sustained and activity-dependent light delivery).

We quantified the waveform change, excitability and circuit sequential dynamics, finding an acceleration of the action potential and the global neural activity in both systems. Also, we analyzed the estimated variation of temperature as a function of the power density applied. We distinguish between temperature and other sources of change and determine the correct combination of power and duration to achieve neuronal modulation.

Our results provide insight into the relationship between laser type, temperature, power, and the resulting excitability effect, and indicate possible mechanisms modulating neural activity beyond a localized temperature increase.

Scale-dependent brain age with higher-order statistics from structural magnetic resonance imaging

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Inferring chronological age from magnetic resonance imaging (MRI) brain data has become a valuable tool for the early detection of neurodegenerative diseases.

We present a method inspired by cosmological techniques for analysing galaxy surveys, utilizing higher-order summary statistics with multivariate two- and three-point analyses in 3D Fourier space. This method identifies outliers while offering physiological interpretability, allowing the detection of scales where brain anatomy differs across age groups and providing insights into brain ageing processes.

Similarly to the evolution of cosmic structures, the brain structure also evolves naturally, but displays contrasting behaviours at different scales. On larger scales, structure loss occurs with age, possibly due to ventricular expansion, while smaller scales show increased structure, likely related to decreased cortical thickness and grey/white matter volume.

Using MRI data from the OASIS-3 database for a sample of ~900 sessions, our method predicts chronological age with a Mean Absolute Error of 4 years (~3.6 years) for individuals aged ~40-100 (50-85), while providing information as a function of scale.

A neural density posterior estimation shows that the 1-σ uncertainty for each individual varies between ~3 and 7 years, suggesting that, beyond sample variance, complex genetic or lifestyle-related factors may influence brain ageing.

This work is part of the CosmicBrain project, demonstrating the utility of interdisciplinary research, bridging cosmological methods and neuroscience.

tDCS Treatment in Patients with Drug-Resistant Chronic Pain, a retrospective study

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INTRODUCTION
An important number of individuals suffering from chronic pain can be considered as drug-resistant patients. In these cases, transcranial direct current stimulation (tDCS) represents a promising and safe complement to medication. A recent evidence-based guidelines paper recommended tDCS over M1 in several chronic NP syndromes [1]. The main objectives of this study were (1) to confirm the safety and efficiency of one-year treatment with tDCS; (2) to calculate the number of "responders" patients to one-year treatment with tDCS; (3) to describe the effects of tDCS on pain intensity during one-year treatment; and (4) to identify factors related to the success of tDCS treatment.

MATERIALS AND METHODS
A total of 155 outpatients were included in the final analysis. Anodal tDCS stimulation on M1 was delivered for 20 min at 1.5mA. The stimulation was in two periods, induction phase (10 session, 2 weeks) and maintenance phase (one-year). An assessment of cognitive and emotional status was performed by MiniMental Status Examination [2] and Beck Depression Inventory [3]. The Visual Analogue Scale (VAS) [4] represents how much pain experience at the moment of completing the scale.

RESULTS
One hundred fifty-five subjects were included in the analysis (mean age: 49.26 ± 11.69 years). An assessment of cognitive and emotional status was performed by MiniMental Status Examination and Beck Depression Inventory. The Visual Analogue Scale represents how much pain experience at the moment of completing the scale.

CONCLUSIONS
The main results were: (1) tDCS is safe; (2) the number of "responders" is approximately 60% of the treated patients; and (3) the maximal pain reduction is achieved after three months. (4) the response to tDCS. patients with a very high VAS at baseline are more likely to not respond adequately to tDCS treatment

Unraveling Neuron-Glia Interactions Using Proximity Ligation Assay: A Novel Approach to Investigate Cellular Responses through Protein Interactions

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The complex interplay between neurons and glia is fundamental for both correct brain function and neurodegeneration. Understanding these interactions at a molecular level remains a key challenge in neuroscience.
Here, we present the use of the proximity ligation assay (PLA) as a powerful tool to visualize in situ protein-protein interactions, allowing us to investigate cellular responses with high spatial resolution. By employing PLA, we can assess organellar proximity and protein aggregation within both neurons and glia, shedding light on dynamic cellular processes that underlie brain function and pathology. The combination of several PLA assays visualizing the interactions between mitochondria (mit), the endoplasmic reticulum (ER) and the lysosome (lys) enables us to distinguish the differential responses of neurons and glial cells when exposed to both physiological and detrimental stimuli, providing a nuanced view of their interactions in health and disease. In addition to this we are able to use PLA in order to track aggregation in neurodegenerative conditions. Our PLA-based findings show that mitochondria to ER and lys proximity is dysregulated in both neurons and astrocytes in genetic forms of Parkinson's Disease (PD), and that this is linked to functional defects such as impaired calcium release. Importantly, through PLA analysis we have also shown that early alpha-synuclein aggregates, such as alpha-synuclein oligomers which can dysrupt interorganellar contacts, accumulate in post-mortem PD samples in both neurons and astrocytes.
This study underscores the potential of PLA as a valuable global tool for neuroscience research, offering a novel way to dissect the molecular crosstalk between neuronal and glial networks.

Viral-mediated fluorescent labeling of hyaluronan reveals extracellular matrix dynamics in the mouse brain in vivo

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The extracellular matrix (ECM) of the brain is primarily composed of the glycan polymer hyaluronan (HA), a core scaffold that nucleates proteoglycans forming a self-assembled matrix that acts as structural framework and signaling hub. Since most of the neural matrix is composed of sugars, development of genetically encoded tags has been limited. Therefore, although several staining protocols exist for ECM in fixed tissue, there are no reliable matrix labels for live imaging. Here we report a viral-mediated fluorescent probe that binds to HA and labels the mouse brain ECM. The vector encodes the HA binding domain from neurocan fused to GFP and an externalization tag (AAV-Ncan-GFP), enabling transduced cells to secrete the fluorescent hyalectan into the extracellular space, thereby labeling HA. We demonstrate stable probe expression in organotypic brain slices, as well as in vivo in the mouse cortex, where it labels both perineuronal nets and interstitial matrix. We validate HA labeling through colocalization with HABP and sensitivity to hyaluronidase, and confirm the probe extracellular localization by shadow imaging. As a proof of concept, we combine AAV-Ncan-GFP with dendritic spine imaging ex vivo and calcium transient imaging in vivo, providing a real-time map of local ECM alongside neural function. The probe enables time-lapse imaging of ECM dynamics in live mice, facilitating longitudinal studies across a wide range of timescales, from minutes to days. The results establish AAV-Ncan-GFP as a valuable tool for real-time observation of brain ECM and a promising resource to explore ECM dynamics and brain function in vivo.

Virtual reality for reducing aggression and its psychophysiological correlates in youth in social conflict. A pilot study

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Recent studies show that Virtual Reality (VR) used in immersive environments can improve intervention in young people in social conflict by reducing violence-related features and early detection of risk factors. However, research in this field is rather scarce.
The study aims to assess the feasibility of a VR intervention to address aggression in young people by designing a structured protocol, validating virtual scenarios, and analyzing key variables.
For this pilot study, young adults aged 18 to 25 are recruited and subjected to a structured protocol in a VR environment, including emotional recognition tasks together with ambiguous and anger situations. Psychological parameters of these individuals are determined by the questionnaires of anger trait and state (STAXI-2), impulsivity (BIS-11), aggression (AQ), reactive and proactive aggression (RPQ), hostile attribution bias (SIP-ABQ, HO), emotion regulation (DERS), alcohol consumption (AUDIT) and cannabis use (CAST). As well, physiological measurements are collected to test participants' responses to VR stimuli. Hormonal biomarkers (cortisol, testosterone and α-amylase) are analyzed from saliva samples before, during and after the VR session. Brain activity via EEG (Muse-2) is also assessed previous, during and after VR exposure, focusing on the prefrontal signal. Heart rate is recorded using Polar H10 to examine cardiac reactivity in response to ambiguous situations and anger.
Preliminary results are presented, expecting the immersive VR experience to induce significant psychophysiological changes. Higher anger, impulsivity and aggression levels are hypothesized to correlate with increased stress and heart rate variability, while hostile attribution bias may lead to similar physiological responses in ambiguous and anger situations. Additional analysis, such as the relationship between emotional regulation and brain activity, is also discussed.
These findings can support the validation of the newly created VR scenarios, provide insights into psychophysiological factors linked to aggression and optimise the use of this tool for future clinical intervention.

HISTORY, EDUCATION AND ETHICS

Mock trial role-play to present scientific papers engage Master students in Neuroscience more effectively and increase their argumentation skills

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Experiential learning strategies, such as role-play and simulations, are debate techniques that actively engage students by encouraging them to discuss and apply course content while getting their maximum participation and implication. These methods promote experience through effective confrontation and the development of new perspectives.
Students in a Master's program in Neuroscience are used to presenting scientific papers during their undergrad years. This study aimed to challenge them into a new way to present a paper by participating in a mock trial where they did not know whether they would play the defence or prosecution attorney role until the same day of the presentation. This required them to use creativity, critical thinking, and implement cognitive flexibility to further get into their assigned role while considering multiple perspectives on the same scientific work.
The satisfaction survey shows that students valued this role-playing activity highly. They perceived that preparing for this activity allowed them to gain argumentative skills and improved their ability to analyze and criticize scientific papers in a constructive manner. Both the ability to defend opposing positions and develop oral communication skills were also highly valued. Overall, the students showed a high level of satisfaction with this activity, highlighting that it had been useful for the development of their academic competences. At the same time, they remarked that this different approach made the preparation of the presentation more interesting and stimulating.
This work is part of the educational innovation project granted by the IRIE (UIB) nº PID242623.
M. Jiménez-García was supported by a FPI grant (FPI_062_2021, Govern de les Illes Balears, Spain).

THE HISTORY OF AUDITORY NEUROSCIENCE IN SPAIN

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This work traces the history of auditory neuroscience in Spain. It began with Santiago Ramón y Cajal, who meticulously described the organization of every region of the auditory system, from the cochlea to the cerebral cortex. Rafael Lorente de Nó then conducted the most detailed study of the cochlear nuclei, and Fernando de Castro later passed the baton to Jaime A. Merchán.

In the 1970s, Jaime Merchán revitalized Spanish auditory neuroscience by attracting to this laboratory three dedicated collaborators. Pablo Gil Loyzaga characterized dopamine and serotonin as key neurotransmitters in the olivocochlear system. José Manuel Juiz and his collaborators in Albacete have investigated the localization of neurotransmitters and their receptors and the pathogenesis and prevention of noise-induced and age-related hearing loss. Juiz also mentored María E. Rubio. Meanwhile, Miguel A. Merchán (Jaime's brother) formed his own group in Salamanca.

Miguel Merchán and his early students (Enrique Saldaña, Dolores E. López, Manuel Sánchez Malmierca and Victoria M. Bajo) studied the anatomy, neurochemistry, and connections of brainstem auditory nuclei. More recently, Miguel Merchán has explored auditory system plasticity and the protective effects of electrical stimulation of the auditory cortex. Victoria Bajo, based in Oxford, focuses on cortical functions and the role of the auditory cortex in learning. Enrique Saldaña specializes on hodology and has discovered several auditory nuclei. Dolores E. López investigates the acoustic startle reflex and audiogenic epilepsy. Manuel S. Malmierca's electrophysiological research on stimulus-specific adaptation is significantly shaping predictive coding theories. The Salamanca Group also includes Enrique López-Poveda, a leading expert in audiology and psychoacoustics.

Today, Spanish auditory neuroscience is in good health, as the followers of Jaime and Miguel Merchán, through their national and international collaborations, are playing a pivotal role in shaping the landscape of the field.

GENERAL NEUROSCIENCE TOPICS

Antibodies against ZSCAN1 in Pediatric and Adult Patients with Non-Paraneoplastic ROHHAD Syndrome

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ROHHAD (rapid-onset obesity, hypothalamic dysfunction, hypoventilation, and autonomic dysregulation) syndrome is a rare disorder characterized by hypothalamic dysfunction, including hyperphagia, sleep apnea, behavioral issues, hypernatremia, and endocrinopathies. It can mimic genetic disorders such as Prader-Willi and congenital central hypoventilation syndrome. Recently, ZSCAN1 antibodies (ZSCAN1-abs) have been identified in pediatric ROHHAD patients associated with neuroblastoma suggesting a role as biomarkers for paraneoplastic ROHHAD.
The aim of this study is to investigate the association between ZSCAN1-abs and patients with ROHHAD syndrome without tumors.
The presence of ZSCAN1-abs was investigated in serum and CSF samples of 13 patients with ROHHAD without tumor, 149 controls with several inflammatory and non-inflammatory conditions and 50 healthy donors by an in-house cell-based assay.
ZSCAN1-abs were detected in 5 of 6 patients (2 female and 3 male) with paired serum and CSF samples available. Four patients tested positive only in CSF, while one was positive in both serum and CSF. Among them, four were children with a median age of 3.6 years (range: 22 months to 7 years), while the other patient was a 58-year-old woman with autoimmune hepatitis who exhibited symptoms of ROHHAD. ZSCAN1-abs were not detected in 7 patients with ROHHAD with only serum available or in any of the 199 control samples.
These findings suggest that patients with ROHHAD should be tested for ZSCAN1-abs in CSF. The presence of these antibodies does not necessarily indicate an underlying tumor, and the disease can also occur in adults.

Astrocyte-Mediated Neurotoxicity in iPSC-Derived Dopaminergic Neurons Induced by TNF-α and IFN-γ

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Parkinson's disease (PD) research has traditionally focused on dopaminergic neurons. However, these cells do not live in isolation in the central nervous system as they are surrounded by astrocytes, amongst other glial cells. Astrocytes display a wide range of functions related to neuronal support and homeostasis that are altered in PD such as the maintenance of the blood-brain-barrier, α-synuclein uptake and degradation, the activation of an inflammatory response, etc.
Here we present data that show a reduced neurosupportive effect of astrocytes in coculture when treated with cytokines (TNF-α and IFN-γ). To this end we generated control and GBA-N370S iPSC-derived ventral midbrain astrocytes (iASTROs) which responded to proinflammatory stimuli as revealed by bulk-RNA sequencing. Interestingly, GBA-N370S iASTROs showed reduced ATP-mediated endoplasmic reticulum (ER)-calcium release when compared to control iASTROs. Moreover, iASTROs displayed a pro-inflammatory phenotype and increased ER-calcium release when treated with TNF-α and IFN-γ. When cocultured together with iPSC-derived dopaminergic neurons (iDAn), cytokine treatment resulted in a reduced neurosupportive effect. Furthermore, cytokine treatment induced α-synuclein preformed fibril (PFF)-mediated neurotoxicity in astrocytes when cocultured with iDAn. These effects were confirmed in an independent co-culture mode, namely a primary mouse midbrain astrocyte-iDAn co-culture.
In line with previous experiments, we investigated the region-specific vulnerability of primary mouse astrocytes (cortex and midbrain) in PD. For this purpose, we treated cortex and midbrain astrocytes with different protein aggregates (α-synuclein and tau) and inflammatory stimulus and studied calcium release and inflammatory phenotype. Preliminary data revealed that midbrain and cortical astrocytes respond differently to α-synuclein and tau in terms of calcium release and inflammatory phenotype. Together this highlights the important role astrocytes play in PD, and by studying their response we may uncover crucial insights for PD therapeutics.

Characterization of the accessory olfactory bulb in Cladistian fishes

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The accessory olfactory bulb (AOB) is a structure located in the rostral telencephalon, anatomically associated with the main olfactory bulb (MOB). Functionally the MOB participates in olfactory processing, while the AOB processes information from the vomeronasal organ. However, the evolutionary origin of this structure is still unknown and its existence in cladistian fishes has not been described. In the present study, first using magnetic resonance imaging (MRI) techniques, followed by neurochemical and connectivity analyses, we studied this anatomical structure in two models of the polypteriform fish group, Polypterus endlicherii and Erpetoichthys calabaricus, key in evolutionary studies to be the group of living fishes closest to the common ancestor of osteichthyan vertebrates. In this model, we used MRI to identify a rostral prominence in the caudolateral part of the olfactory bulb, which receives independent afferents from the main olfactory nerve. Furthermore, the conserved neurochemical pattern of this structure, as well as its connectivity with amygdaloid-like telencephalic structures, identified based on its genoarchitecture and neurochemistry, strongly supports the existence of a functional AOB in the brain of P.endlicherii and E.calabaricus.

Comorbidity mechanisms of depression in Parkinson's disease and its effects on dopaminergic and serotonergic populations of Dorsal Raphe Nucleus

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In this study, we examine neuropathological features in the dorsal raphe nucleus (DRN) of Parkinson's disease patients with and without anxiety and depression (PDAND and PD, respectively). Immunohistochemistry revealed distinct distributions of dopaminergic (TH+) and serotonergic (TPH2+) neurons along the DRN's rostro-caudal axis. Both PD and PDAND patients exhibited severe loss of TH+ neurons in the DRN compared to controls. Specifically, in the caudal region of the DRN, PDAND patients showed greater reduction in dopaminergic neurons compared to PD patients, potentially linked to a higher alpha-synuclein (paSyn) burden in TH+ neurons of PDAND patients. Interestingly, PDAND TH+ neurons showed fewer Lewy bodies (LB) and more diffuse paSyn distribution, suggesting a possible protective role of LB. Conversely, serotonergic neurons in both PD and PDAND patients accumulated paSyn, but the total number of Tph2+ neurons remained similar to controls. Overall, our findings underscore the complex pathology of DRN neurons in Parkinson's disease and highlight differential vulnerability to alpha-synuclein pathology between dopaminergic and serotonergic populations.To address possible origins for the higher neurodegeneration in the patients with comorbidities, panmicroglial marker Iba1 and active microglia markers CD 68 and MHCII were observed to assess the differences in the level of neuroinflammation. The mean intensity of the proinflammatory biomarker CD68 was significantly higher in the DRN of PDAND patients compared to PD patients and controls, with PD patients also showing greater CD68 mean intensity than controls. The MHCII marker showed a greater microglial activation in patients with comorbidities than in the control patients, with a higher glia colocalization ratio in PD patients. A morphological analysis was performed to asses difference in morphology of the microglia, revealing an exclusive and intense dystrophy in PD patients. All these results point to a differentiated inflammatory degeneration pattern associated to Parkinson's disease patients that also suffer from depression.

Cortical type: a theoretical framework for the biological interpretation of epigenetic regulation, glutamatergic, and GABAergic gene expression data in the human cerebral cortex

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Contemporary science is characterized by the acquisition of massive datasets obtained with high-throughput gene expression techniques. The interpretation of this massive data poses a challenge as it requires a theoretical framework that provides biological meaning. In this study, we present an example of the interpretation of massive data within a biologically meaningful theoretical framework. Specifically, we analyse and interpret large-scale transcriptomic data from the human cerebral cortex within the framework of the laminar complexity gradient. The proposed hypothesis is that the expression of genes related to glutamatergic transmission and epigenetic regulation will be higher in cortical regions with lower laminar complexity, whereas the expression of genes related to GABAergic transmission will be higher in regions with greater complexity. Gene expression data were extracted from the Allen Brain Institute database, which contains data from the brains of six neurotypical donors and one hundred donors with Alzheimer's disease. In the case of neurotypical brains, 44 genes were analysed, of which 10 matched the predictions, 1 contradicted them, and 31 showed no pattern. In the case of brains with Alzheimer's, 42 genes were analysed, of which 25 matched the predictions, 1 contradicted them, and 16 showed no pattern. In conclusion, the cortical gradient of laminar complexity serves as a valuable theoretical framework for the biological analysis and interpretation of large-scale gene expression data in the human cerebral cortex. This framework can help design experiments to study neurological diseases.

DETERMINING THE PROTEIN INTERACTOME OF LARGE CONDUCTANCE VOLTAGE- AND CALCIUM-GATED CHANNELS IN NEURONAL CALCIUM NANODOMAINS

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The large conductance voltage- and calcium-gated potassium channels (BK) are widely expressed in the central nervous system. BK activation relies on changes of the membrane voltage and on the local influx of Ca 2+ to achieve micromolar levels. In many neuronal types, they establish functional associations with calcium sources such as NMDA receptors (NMDAR) located in close proximity (<50 nm). The study
of the NMDAR-BK complexes was originally focused on hippocampal and cortical neurons. In the latter, we have proposed that NMDAR-BK multichannel complexes serve as high-pass filters for incoming synaptic inputs (e.g. Gomez et al 2021, PMID: 34453004). Nonetheless, growing evidence (including work from our laboratory) demonstrates that this mechanism is present in diverse neuronal types and may have
been overlooked in many physiological settings (we have reviewed this topic recently in Martinez-Lazaro et al 2025, PMID: 39874044).

To understand how these macrocomplexes are organized and regulated, we are now studying the full protein interactome of BK and NMDAR using the Bio-ID technique. To this end, we use cell lines of neuronal origin expressing BK and NMDAR. We aim to assess the composition of these macrocomplexes interactomes in control and pathophysiological conditions.
This study brings new data on the existence of novel BK partners and regulatory mechanisms that modulate the function of multichannel complexes involved in synaptic
plasticity.

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Distinct antibody glycosylation profiles reflect immune response compartmentalization and disease triggers in anti-NMDAR encephalitis

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Anti N-Methyl-D-Aspartate Receptor encephalitis (NMDARe) is a severe neurologic disorder characterized by pathogenic antibodies targeting the NMDAR. Disease triggers include ovarian teratoma, or herpes simplex virus encephalitis (HSE), though in many cases the cause is unknown (idiopathic). While NMDAR antibodies, primarily of the IgG1 subclass, are often detected in serum and CSF, some patients have antibodies only in CSF. This, along with the presence of CSF-unique IgG oligoclonal bands, suggests a compartmentalized antibody response within the brain compared to the periphery. Whether these antibodies differ qualitatively between serum and CSF or based on disease trigger is unclear. Here, we aimed to characterize serum and CSF antibodies in patients with anti-NMDARe by analyzing their Fc-glycan composition, and assess whether different glycosylation profiles could be identified based on disease triggers.
Paired serum and CSF samples from age- and sex-matched patients with anti-NMDARe (n=50) were analyzed using liquid-chromatography-mass-spectrometry to determine their IgG1 antibody-glycosylation profiles, including quantification of fucose, sialic acid, galactose and bisecting N-acetylglucosamine. These glysosylation profiles were then compared between compartments (CSF vs serum) and among different triggers (post-HSE, tumor-related, idiopathic).
Compared to serum, CSF antibody glycans exhibited lower levels of sialic acid and galactose, indicating a more inflammatory profile. Similarly, patients with post-HSE NMDARe displayed an enrichment of inflammatory glycoforms, characterized by reduced sialic acid and galactose levels and increased bisecting N-acetylglucosamine, compared to those with tumor-related or idiopathic causes.
In conclusion, our data demonstrate that antibody glycan profiles in patients with anti-NMDARe are compartmentalized, with CSF exhibiting more inflammatory glycoforms than serum. Furthermore, glycan composition varies by trigger, with post-HSE anti-NMDARe linked to more inflammatory glycovariants than other causes. These findings highlight the role of glycans in the pathogenesis of NMDARe, which could be harnessed to develop novel compartment- and trigger-specific therapeutic strategies based on glycan modifications.

Effects of Video-Guided Active Breaks on Motor Competence, Language development and Self-esteem of School Children with Special Education Needs: The Active Classes Project

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Aim: To assess the effects of a video-guided active break program on motor competencies, language, and self-esteem in schoolchildren with special educational needs (SEN).
Methods: A controlled trial was conducted involving 161 schoolchildren (mean age 7.8 ± 1.1 years) with SEN from public schools. Participants were assigned to either a control group (n=85), which did not receive active breaks, or an experimental group (n=76), which participated in the active break program. The intervention consisted of a 12-week video-guided active break program implemented twice daily, five days per week, via a web-based platform. Motor competencies were evaluated using the MOBAK tests, language skills were assessed using the Comprehensive Learning Diagnosis, and self-esteem was the School Self-Esteem Test.
Results: Significant improvements were observed in all motor competencies in the experimental group. The time × group interaction effect was significant, with improvements ranging from 7.6% to 20.4%. Regarding language, significant time × group interactions were found for locating ($F(1,154) = 10.239$, $p = 0.002$, $\eta^2 = 0.012$) and reflecting ($F(1,154) = 28.663$, $p < 0.001$, $\eta^2 = 0.067$). A significant time effect was found for self-esteem, showing an improvement in both groups post-intervention.
Conclusions: The video-guided active break program significantly enhanced motor competencies, language skills, and self-esteem in schoolchildren with special educational needs in the experimental group compared to the control group. These findings suggest that school-based, guided active break programs may be an effective strategy for promoting health and development in schoolchildren with SEN.
link to video summary: https://www.youtube.com/watch?v=5W6jMNnsCWk&t=12s
Web: https://clasesactivas.cl/auth/signin?callbackUrl=%2F

Hierarchy and Destiny of Mitochondrial Transfer Between Neurons and Glial Cells

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The interplay between neural cells, particularly regarding mitochondrial composition and metabolic communication, is a highly coordinated event. The interaction between neurons and astrocytes plays a pivotal role in both the physiology and pathology of the nervous system, with mitochondrial function being essential for maintaining homeostasis and cellular viability. In this scenario, the acquisition of extracellular mitochondria and the transfer of mitochondria between neural cells, are consolidating as key players in the intercellular communications that shape mitochondrial content and function in the Nervous System.

Here we report that neurons and astrocytes acquire exogenous or transferred mitochondria. Differential avidity, fate, and persistence of the imported organelles or their genetic material (mtDNA) are revealed in both neural cell types through microscopy, flow cytometry, and RT-PCR analyses. We show that the fate of the imported mitochondria results from the balance between their integration into the recipient mitochondrial network and their subsequent disposal through selective mitochondrial degradation, as revealed by a mitophagy reporter mouse model (MitoQC).

Notably, mitochondrial transfer between neurons and astrocytes exhibits hierarchical patterns of acquisition in vivo, as revealed by viral delivery of genetically encoded mitochondrial markers under specific neuron or astrocyte promoters. In line with this, different brain regions act as preferred sites for the fate of exogenous mitochondria systemically administered by retro-orbital injections, potentially influenced by microglia. In summary, mitochondrial transfer between neural cells is a finely tuned event crucial for shaping their content and physiology in the nervous system.

How Similar are the Pallial Fields of Ray-Finned Fishes? Comparative Organization of Telencephalic Regions in a Basal and a Derived Actinopterygian Fish

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The present study compares the telencephalic regions of two key actinopterygian models. As a basal model, we chose the bichir Polypterus senegalus, a cladistian fish phylogenetically close to the common osteichthyan ancestor. One of its most interesting features is the presence of an open-everted pallium with a pseudoventricle. As a derived model, we used Carassius auratus, a cyprinid teleost with great pallial complexity due to secondary fusion of its everted pallium. In this work, based on the analysis of their genoarchitecture, neurochemistry and connectomic, we revealed the similarities and differences between the main telencephalic territories in a rostrocaudal progression based on the distribution of highly conserved markers and their functional connectivity. The territories analyzed included the ventral telencephalic area in the subpallium and the dorsomedial and dorsolateral pallial areas in the pallium, which are rostrocaudally and functionally distinct in both models, as well as the putative amygdaloid territories of the dorsoventral pallium and posterior pallium in C. auratus and the supracommissural and postcommissural territories in P. senegalus. Our data provide evidence for a common ancestral telencephalic pattern with specific anatomical and functional features in each model.

IMPACT OF THE LRRK2 G2019S MUTATION ON ALPHA-SYNUCLEIN OLIGOMER ACCUMULATION AND MICROGLIAL ACTIVITY IN PARKINSON'S DISEASE

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The G2019S mutation in the leucine-rich repeat kinase II gene (LRRK2) is a major cause of familial Parkinson's Disease (PD), a complex neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). This mutation enhances the activity of the LRRK2 protein, leading to increased aggregation of α-synuclein (α-syn), potentially accelerating PD pathology. Recent studies in PD animal models have highlighted the role of neuroinflammation in disease progression, with evidence of heightened brain inflammation mediated by microglia in both animal and human carriers of G2019S LRRK2.

This research is focused on understanding how the G2019S LRRK2 mutation influences the accumulation of α-syn oligomers, as well as microglial activity and response in the SNc, striatum, hippocampus, motor cortex, anterior olfactory nucleus, and medulla oblongata of male and female LRRK2 wildtype and G2019S LRRK2 knock-in mice, aged 12 or 48 weeks. Immunohistochemistry and Proximity Ligation Assay (PLA) results have revealed a complex interplay between gender, LRRK2 genotype, and age leading to changes, on the one hand, in the number and distribution of α-syn oligomers in the brain, and, on the other hand, in the microglial phenotype, where parameters such as the phagocytized number of oligomers by the microglia and cell morphology happen to be altered.

Overall, this study demonstrates the interplay between age, gender, and LRRK2 genotype in the regulation of the number and distribution of α-syn oligomers and in the microglia response regarding α-syn oligomeric neuroinflammation of different brain areas that are shown to be affected in PD.

KAT3 proteins maintain cell identity transcriptional programs in distinct neural lineages

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Cellular identity in neural lineages is acquired thanks to the coordinated induction of terminal selectors and complex chromatin rearrangements, allowing for the expression of the necessary transcriptional programs required for functional specification. Once stablished, cellular identity is actively maintained, thanks in a big extent to the self-regulation of terminal selectors, but also to additional molecular mechanisms contributing to this process. Several chromatin modifiers are thought to present a crucial role in this maintenance. For instance, the combined elimination of the lysine acetyl-transferase type 3 proteins CBP and p300 leads to a rapid and dramatic loss of neuronal identity in post-mitotic excitatory neurons of the adult mouse brain. It is not clear, however, the molecular and physiological dynamics taking place immediately after their elimination in neurons and the implication of these proteins in the cellular identity maintenance of other neural lineages. By taking advantage of a recently developed KAT3-specific PROTAC degrader, we interrogated the transcriptional and physiological dynamics resulting from the acute elimination of KAT3 proteins in neuronal primary cultures. Our analyses unveil the sequence of molecular processes that precede the shutdown of specialized neuronal transcriptional programs. Additionally, we also generated a time-series of RNA-seq data in glial primary cultures, showing that the proposed role of KAT3 proteins preserving cell identity-specific transcription is not restricted to neuronal cells. Glial cells show a similar progression towards cellular identity loss with lineage-specific gene sets affected when compared to identity loss in neuronal primary cultures. Our data suggests that KAT3-mediated cellular identity maintenance is a conserved mechanism among distinct neural lineages executed thanks to the action of lineage-specific terminal selector transcription factors. Despite that, transcriptional signatures identified in glial and neuronal primary cultures present a substantial level of overlap, suggesting the existence of conserved transcriptional pathways regulated by KAT3 proteins in distinct neural lineages.

Localization of the Nkx2.2 expression pattern in the brain of Polypterus, the most basal actinopterygian fish

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The transcription factor Nkx2.2 is involved in the control of the cell fate and regional patterning in the ventral neural tube (closely related to Sonic hedgehog), the establishment of the alar-basal boundary, and the specification of the diencephalic domain. Its expression pattern has been studied thoroughly in the forebrain of tetrapods, since it is a useful tool for the regionalization of this area, but there is a striking lack of information concerning the hindbrain distribution and its localization in the brain of actinopterygian vertebrates. Among these, cladistian fishes are the most basal group and the closest to the common ancestor of all osteichthyan vertebrates, which makes them highly suitable models for the study of the evolution of the central nervous system. In order to study the expression pattern of this transcription factor in cladistians, we performed immunohistochemical techniques for the detection of Nkx2.2, together with the transcription factors Orthopedia and Pax6, and tyrosine hydroxylase, in the brain of the Senegal bichir, Polypterus senegalus, a representative species belonging to one of the two genera of cladistian fishes. We observed immunoreactivity in cell nuclei of the caudal portions of the paraventricular and subparaventricular hypothalamic domains, the tuberal and mamillary domains within the basal hypothalamus, prethalamus, thalamus, basal portion of the first diencephalic prosomere, and the mesencephalic tegmentum. These results show a high degree of conservation in the expression pattern of Nkx2.2 in many of these regions.

Metabolic dysregulation and reactive astrogliosis in cerebellar astrocytes of a Friedreich´s ataxia murine model

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Friedreich's ataxia is an autosomal recessive neurodegenerative disorder characterized by a deficiency in the mitochondrial protein frataxin, resulting from an expanded GAA trinucleotide repeat within the first intron of the FXN gene. This frataxin deficiency leads to progressive degeneration of the cerebellum, dorsal root ganglia, and peripheral nerves, manifesting clinically as impaired coordination (ataxia) and loss of balance. Recent investigations have expanded our understanding of Friedreich´s ataxia pathophysiology beyond its primary neuronal impact. Emerging evidence suggests a significant role for non-neuronal cells in the disease process, with particular emphasis on astrocytes. This study aimed to characterize the cerebellar astrocyte pathophysiology, investigate astrocyte-neuron interactions and test potential therapeutic interventions using primary astrocytes derived from the YG8-800 (Fxnnull::YG8s(GAA)>800) mouse model. This humanized Friedreich´s ataxia model is homozygous for a Fxn constitutive null allele and hemizygous for a human FXN transgene with >800 GAA repeats, that exhibits reduced frataxin levels, relative to control mice (Y47R), which express human FXN with only 9 GAA repeats. We characterized the reactivity phenotype, mitochondrial function and induced neurotoxicity of YG8-800 and Y47R-derived primary cerebellar astrocytes obtained from 5-day-old pups. When compared to control cells, cerebellar astrocytes from ataxic mice show significant mitochondrial and metabolic alterations that were accompanied by a shift in their reactivity profile that ultimately caused damage to cerebellar granular primary neurons through conditioned media. Furthermore, we evaluated the effects of the long-term treatment with SAG, a Sonic hedgehog pathway agonist with modulatory effects on astrocytes. We found that it effectively reduced astrocyte activation and its associated neurotoxic effects. Collectively, our findings suggest that modulation of the sonic hedgehog pathway in astrocytes could mitigate some of the altered processes observed in YG8-800-derived cells.

Neuroscience applied to team sport. Socio-affective implications in the sport of rowing in adolescents

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When performing physical exercise, not only conditional (strength, speed, endurance, etc.), coordinative (movement control, implementation of movement in the appropriate space and time, etc.) and cognitive (stimulus identification, visual attention, decision making, etc.) capacities must be considered, but also those involving intrapersonal (stress, satisfaction, anxiety, boredom or enjoyment, etc.) and interpersonal (cooperation, competition, acceptance/rejection of partner or rival, etc.) relationships. The Ringelmann effect is a phenomenon that shows a decrease in performance as group size increases due to lack of coordination and social loafing (Ingham et al., 1974). Conversely, due to the Köhler effect, the lower performing teammate at the individual level performs better as they feel that their individual effort is relevant to the success of the team (Köhler, 1926, 1927; Hertel et al., 2018). Likewise, while team sports require greater involvement of the limbic system, especially the amygdala and the orbitofrontal cortex, individual sports require more decision-making and planning, involving the prefrontal cortex to a greater extent. In this study we analysed whether there were differences in the performance of adolescent rowing athletes when performing an individual or group test. The results showed differences in the perception of effort depending on age. While 16–17-year-old rowers show a greater subjective perception of effort when rowing individually than when rowing in a team, this effect was not observed in 14–15-year-olds. The results obtained suggest that the differences found at different ages may be due to the different degree of development of the prefrontal cortex, as this is involved in the cognitive interpretation of effort and in the regulation of behaviour in the face of fatigue.

Non-Invasive Classification of Mental Health Disorders Using Resting-State EEG with Dry Electrodes for Scalable Triage

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The purpose of this study is to develop a model utilizing resting-state EEG data acquired through dry electrodes to classify individuals with and without mild psychopathological symptoms for triage purposes. The proposed methodology uses a Random Forest model. Coherence connectivity was identified as the most predictive feature, achieving an accuracy of 100% in distinguishing between epochs. The classifier is then applied to the sequences of epoch-level predictions by file, demonstrating file-level classification, achieving an accuracy of 86,34% with test files. These findings highlight the potential of non-invasive, dry electrode EEG and Machine Learning approaches as scalable tools for mental health disorder classification and clinical triage.

Purinergic Receptor P2Y13 Controls Activation and Mode of Division in Subependymal Adult Neural Stem Cells

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The subependymal zone (SEZ) of the mammalian brain is the most active germinal area that continues to generate newborn neurons throughout life. This area harbors a population of neural stem cells (NSCs) that can be found in different states of activation, each differing in proliferative capacity and molecular signature: quiescent NSCs (qNSCs), primed NSCs (pNSCs), and activated NSCs (aNSCs). There is currently a void in terms of the specific markers available to effectively discern between these transient states. Likewise, the molecular signaling mechanisms controlling the transition from quiescence to activation remain largely unexplored, as do the factors influencing the decision between differentiation and self-renewal during NSC division. Here, we present evidence that the metabotropic P2Y13 purinergic receptor plays a critical role in regulating adult neurogenesis. We found that P2Y13 is specifically expressed in NSCs within the adult SEZ and that its levels can be used to distinguish qNSCs from aNSCs. Functionally, P2Y13 signaling promotes NSC activation, enhancing lineage progression, while dampening their self-renewal capacity. Conversely, pharmacological blockade or genetic silencing of the P2Y13 receptor favors NSC quiescence. Thus, we identified the metabotropic P2Y13 purinergic receptor as a pivotal modulator of NSC dynamics, influencing both the balance between NSC quiescence and activation and the mode of NSC division at the subependymal zone.

Role of RBPMS-Associated RNAs Under Hypoxia and Neurodegeneration in Retinal Ganglion Cells

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Retinal ganglion cells (RGCs) transmit visual information to the brain, relying on precise RNA regulation mediated by RNA-binding proteins such as RBPMS, which is selectively expressed in these cells. While previous studies have identified changes in RBPMS distribution in RGCs under stress, the molecular mechanisms underlying these changes remain poorly understood. This study analyzes RBPMS-associated RNA under hypoxic and neurodegenerative conditions, providing insights into its role in RGCs and offering targets for therapeutic intervention in retinal diseases.

Porcine retinas were used, and to simulate hypoxia, enucleated eyes were stored at 4°C for 24 hours before retinal dissection. For the neurodegeneration model, retinal explants were cultured in vitro for three days under controlled conditions. RNA associated with RBPMS was isolated via immunoprecipitation using magnetic beads specific to RBPMS, followed by RNA extraction. The extracted RNAs were analyzed using RNA-sequencing (RNA-seq) to identify transcripts bound to RBPMS. ASPeak (abundance-sensitive peak detection algorithm) was employed to analyze and to compare all conditions from RNA-seq data.

Functional analysis revealed that the most relevant categories were transcriptional regulation (28.40%), mitochondrial metabolism (22.07%), growth and development (10.76%), and cell signaling (9.19%). Interestingly, the enrichment of mitochondrial metabolism-related transcripts underscores the potential role of RBPMS in modulating mitochondrial activity during hypoxia and neurodegeneration. These findings suggest that RBPMS regulates RNA networks critical for mitochondrial function, contributing to cellular adaptation under stress.

This study provides novel insights into the role of RBPMS in RGCs, regulating RNA networks associated with mitochondrial function under hypoxic and neurodegenerative conditions. This identifies RBPMS as a potential therapeutic target for preserving mitochondrial health in neurodegenerative ocular disorders like glaucoma.

Sex-dependent effects of ibogaine on gene expression in the mouse brain: A transcriptomic study of therapeutic potential

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Opioid overdose is a significant public health crisis that causes thousands of deaths each year. Ibogaine (IBO), a psychoactive alkaloid, shows potential in treating drug dependence and mitigating withdrawal symptoms, but its mechanism of action remains unclear. This study examined IBO-induced changes in gene expression in male and female C57BL/6 mice. Adult mice were administered by a single oral dose of 0 or 60 mg/kg of IBO by gavage. Four hours post-administration, frontal cortex samples were collected from three animals of each sex and treatment for transcriptomic analysis. In parallel, four and eight hours after IBO administration, six animals of each sex and treatment were euthanized to obtain samples from the frontal cortex, striatum and hypothalamus for qPCR. Animals were also assessed using the Functional Observational Battery scale to evaluate possible adverse effects of the treatment. Transcriptomic analysis revealed that IBO increased the expression of genes related to oxytocin, vasopressin and synaptogenesis, while genes associated with apoptosis and endosomal transport were reduced. Gender differences were observed, with up to 28 genes affected in females and eight in males. Gene expression at four and eight hours showed IBO effects in all the brain areas evaluated, while sex differences in hormonal pathways and synaptogenesis were observed in hypothalamus and striatum. Our result indicates that after the IBO peak at four hours its impact on gene expression persists or even increases. This study expands our understanding of IBO molecular action. Further research is needed to investigate the role of IBO and its metabolite Noribogaine in the changes and to assess the long-term effects of IBO.

α-Synuclein neuronal overexpression and spread to astrocytes impairs mitochondrial function in vitro in both cell types differentially.

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Dopaminergic neuronal loss is the main pathological hallmark of Parkinson's disease (PD), the most common neurodegenerative movement disorder worldwide, mainly due to the intracellular aberrant inclusions of the α-synuclein protein (α-SYN). Strong evidence suggests that its accumulation at mitochondrial level induces mitochondrial fragmentation, increased oxidative stress, reduced ATP production and overall mitochondrial dysfunction and ultimately neuronal death. In addition, although recent studies indicate the extracellular presence of α-SYN in the PD context, it is not yet well known how it could affect neighboring cells such as astrocytes nor their mitochondria. This would be of extreme importance since astrocytes provide structural and metabolic support to neurons. In this work, we focused on the neuron-astrocyte crosstalk, analyzing the functional diversity induced by α-SYN in mechanisms associated with mitochondrial activity in both cell types. To this aim, using transwell inserts we co-cultivated α-SYN over-expressing mouse neuroblastoma cell line N2a (N2a-α-SYN) with immortalized mouse astrocytes (IMA 2.1) for 48h, creating a α-SYN enriched media. We identified that, compared to N2a WT, N2a-α-SYN show a differential expression pattern of RNA and proteins levels related to mitochondrial function such as related to the electron transport chain, to the ATP synthase , and the correct folding of imported proteins , and related to mitochondrial dynamics (fission and fussion). Furthermore, astrocytes also show variance on the levels of mentioned proteins depending on the presence or absence of neuron-derived α-SYN in the media. These results conclude that different pathways and mechanism of action are activated on the presence or absence or of α-SYN and cellular type, opening new therapy targets and strategies for treatment of the disease