XIII SIMPOSI DE NEUROBIOLOGIA

Complexitat cel·lular en la funció i disfunció cerebral

28 i 29 de Maig de 2024
Institut d'Estudis Catalans, Barcelona
Programa i resums de les comunicacions

Amb el patrocini de:

Amb la participació de:

COMITÈ ORGANITZADOR / SECRETARIA TÈCNICA / LOCALITZACIÓ

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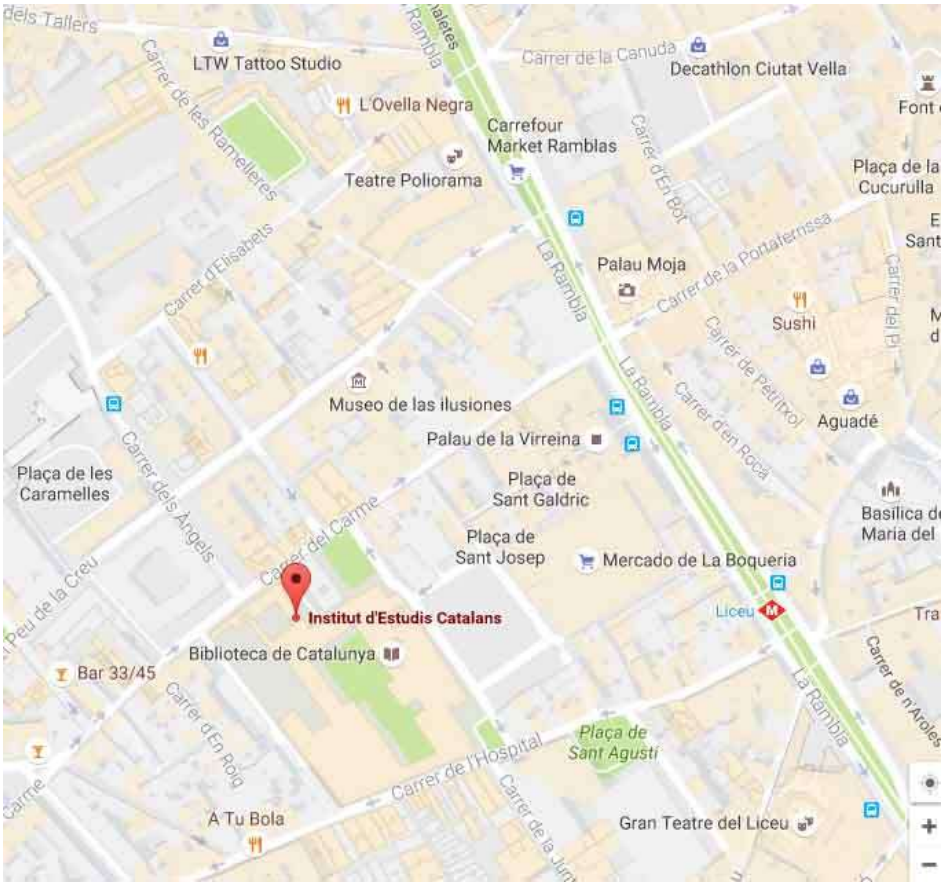

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PROGRAMA

Dia 1: 28 Maig de 2024		Dia 2: 29 Maig de 2024	
8.30-9 h	**Registre i recollida de material**		
9-9.15 h	**Benvinguda i presentació** Sala: Prat de la Riba		
9.15-11h	**Sessió 1A** Oral **Cèl.lules glials i inflammació** Sala: Prat de la Riba **Sessió 1B** Oral **Neurodesenvolupament i malalties relacionades** Sala: Pere i Joan Coromines	9-10.45 h	**Sessió 3A** Oral **Circuits neuronals i plasticitat cerebral** Sala: Prat de la Riba **Sessió 3B** Oral **Sistemes motor i sensorial** Sala: Pere i Joan Coromines
11-12 h	Cafè i sessió de **Pòsters**	10.45-12 h	Cafè i sessió de **Pòsters**
12-13 h	**Conferència plenària 1** Sala: Prat de la Riba **Prof. Isabel Fariñas, Universitat de València-CIBERNED** «The impact of neural stem cell heterogeneity on niche dynamics»	12-13 h	**Conferència plenària 3** Sala: Prat de la Riba **Getrudis Perea, Instituto Cajal-CSIC** «Neuron-astrocyte signaling in stress-induced depressive-like states»
13-15h	**Dinar i sessió de pòsters**	13-15 h	**Dinar i sessió de pòsters**
15-16.45 h	**Sessió 2A** Oral **Malalties neurodegeneratives I** Sala: Prat de la Riba **Sessió 2B** Oral **Receptors de neurotransmissors i senyalització** Sala: Pere i Joan Coromines	15-16.45 h	**Sessió 4A** Oral **Malalties neurodegeneratives II** Sala: Prat de la Riba **Sessió 4B** Oral **Cognició i desordres mentals** Sala: Pere i Joan Coromines
16.45-17.45 h	**Conferència plenària 2** **Dr. Laurent Groc, CNRS/Bordeaux Université** Sala: Prat de la Riba «Exploring brain cell communication at the single molecule level in health and disease: learning from the NMDA receptor»	16.45-17.45 h	**Conferència plenària 4** **6th Ramón Turró Award** Sala: Prat de la Riba **Prof. Eduardo Soriano, Universitat de Barcelona, CIBERNED** «The 1996/1998 breaktrough: from Reelin and Cajal-Retzius cells to adult plasticity and Alzheimer´s disease»
17.45-19.30h	**Refrigeri i sessió de pòsters**	17.45-18 h	**Premis i Clausura**
		18-20h	**FESTA DE CLAUSURA**

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Day 1: May 28th 2024		Day 2: May 29th 2024	
8.30-9 h	**Registration**		
9-9.15 h	**Welcome presentation** Room: Prat de la Riba		
9.15-11h	**Session 1A** Oral **Glial cells and inflammation** Room: Prat de la Riba **Session 1B** Oral **Neurodevelopment and related diseases** Room: Pere i Joan Coromines	9-10.45h	**Session 3A** Oral **Neural circuits and brain plasticity** Room: Prat de la Riba **Session 3B** Oral **Motor and sensoty systems** Room: Pere i Joan Coromines
11-12 h	Coffee and **Poster session**	10.45-12h	Coffee and **Poster session**
12-13 h	**Plenary Lecture 1** Room: Prat de la Riba **Prof. Isabel Fariñas, Universitat de València-CIBERNED** «The impact of neural stem cell heterogeneity on niche dynamics»	12-13h	**Plenary Lecture 3** Room: Prat de la Riba **Getrudis Perea, Instituto Cajal-CSIC** «Neuron-astrocyte signaling in stress-induced depressive-like states »
13-15h	**Lunch and poster session**	13-15 h	**Lunch and poster session**
15.00-16.45 h	**Session 2A** Oral **Neurodegenerative diseases I** Room: Prat de la Riba **Session 2B** Oral **Neurotransmitter receptors and signalling** Room: Pere i Joan Coromines	15.00-16.45h	**Session 4A** Oral **Neurodegenerative diseases II** Room: Prat de la Riba **Session 4B** Oral **Cognition and mental disorders** Room: Pere i Joan Coromines
16.45-17.45 h	**Plenary Lecture 2** **Dr. Laurent Groc, CNRS/Bordeaux Université** Room: Prat de la Riba «Exploring brain cell communication at the single molecule level in health and disease: learning from the NMDA receptor»	16.45-17.45h	**Plenary Lecture 4** **6th Ramón Turró Award** Room: Prat de la Riba **Prof. Eduardo Soriano, Universitat de Barcelona, CIBERNED** «The 1996/1998 breaktrough: from Reelin and Cajal-Retzius cells to adult plasticity and Alzheimer´s disease»
17.45-19.30h	**Drinks and poster session**	17.45-18h	**Awards and Closing**
		18-20h	**CLOSING PARTY**

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DAY 1: Tuesday, May 28th 2024

8.30-9h	**REGISTRATION**
9-9.15h	**WELCOME PRESENTATION** *Prat de la Riba Room* Organizing Committee
9.15-11h	**ORAL SESSIONS** **Session 1A. GLIAL CELLS AND INFLAMMATION** *Room: Prat de la Riba* **Chair: Mar Puigdellívol (Institut de Neurociències, Universitat de Barcelona; Institut de Neurociències-Universitat Autònoma de Barcelona)** **O.1.** Cannabidiol breaks the chains placed by A2AR on cannabinoid receptor 1 in ischemic stroke **Raïch I** **O.2.** Phenotypic characterization of lipid droplet-accumulating microglia after cerebral ischemia **Pedragosa J** **O.3.** Intravenous gene therapy provides long-term correction of cerebral white matter swelling and locomotor deficiencies in a mouse model of megalencephalic leukoencephalopathy with subcortical cysts. **Brao A** **O.4.** Cannabidiol extends lifespan and improves clinical signs in a mouse model of leigh syndrome. **De Donato MH** **O.5.** Astrocytic RTP801 is involved in neurodegeneration and neuroinflammation in alzheimer disease. **Chicote-Gozález A** **O.6.** The gut-brain axis in a novel humanized transgenic mouse model for Parkinson´s disease and brain aging. **Lorente-Picón M** **O.7.** Elucidating the role of ATF3 in the neuropathology of a mouse model of leigh syndrome. **Blanco-Ramos M** **Session 1B. NEURODEVELOPMENT AND RELATED DISEASES** *Room: Pere i Joan Coromines* **Chair: Joaquim Egea (IRB Lleida, Universitat de Lleida)** **O.8.** CXCL14 is a key chemokine involved in axon guidance of pioneer axons of the statoacoustic ganglion. **Rumbo M** **O.9.** Astrotactin 1 deficiency results in a novel neurodevelopmental disorder. **Gatnau-Civardi** **O.10.** 4D-study of neuronal cell lineages and the generation of neuronal diversity in the zebrafish hindbrain. **Ortiz-Álvarez G** **O.11.** The hidden side of NCAM family: NCAM2, a key cytoskeleton organization molecule regulating neuronal differentation and synaptic formation in brain development. **Parcerisas A**

	O.12. The role of the RNA-binding protein staufen 2 during neurogenesis. **Fernández Moya SM** **O.13.** Ketogenic diet as a potential treatment for SPATA5-related encephalopathy: the power of mitochondrial modulation in the treatment of neurodevelopmental diseases. **Musokhranova U** **O.14.** Novel strategies to decrypt the complexity of growth cone responses: from signal integration to motile responses. **Ros O**
11.00-12.00	**COFFEE BREAK and POSTERS**
12.00-13.00	**PLENARY LECTURE** *Prat de la Riba Room* Presented by: S. Ginés **«The impact of neural stem cell heterogeneity on niche dynamics»** Isabel Fariñas (Universitat de València-CIBERNED)
13.00-15.00	**LUNCH**
15.00-16.45	**ORAL SESSIONS** **Session 2A. NEURODEGENERATIVE DISEASES I** *Prat de la Riba room* **Chair: Gemma Navarro (Institut de Neurociències, Universitat de Barcelona)** **O.15.** Role of NR2A and NR2B on the neuroprotective effects of CB1R in Alzheimer's disease. **Rebassa JB** **O.16.** Cell-type specific hippocampal alterations are associated with memory deficits in novel Alzheimer's disease transgenic mice.**Deprada A** **O.17.** Evaluation of the functional activity in synaptic genes related to polygenic risk for Alzheimer's disease using a massively parallel reporter assay. **Perlaza D** **O.18.** Single-cell transcriptomics analyses unveil post-transcriptional regulation alterations in iPSC-derived neuron and glia cells from Alzheimer's disease patients. **Gutiérrez-Franco A** **O.19.** Pathological interplay between alpha-synuclein and neuromelanin accelerates Parkinson's disease pathology in melanized rodents. **Nicolau-Vera A** **O.20.** Deciphering serotonergic synaptic alterations in synucleionopathy and depression copathology mouse model**. Sarriés-Serrano U** **O.21.** The uncharted non-coding layer of the human transcriptome reveals potential new players in brain (patho)physiology. **Perteghella T**

	Session 2B. NEUROTRANSMITTER RECEPTORS and SIGNALING *Pere i Joan Coromines Room* **Chair: Alex Bayés (Institut de Recerca, Hospital Santa Creu i Sant Pau)** **O.22.** Synaptic proteome diversity is primarily driven by gene regulation of glutamate receptors and their regulatory proteins. **Bayés À** **O.23.** Mouse cortical astrocytes detect dopamine via non-cognate receptors. **Pittolo S** **O.24.** In vivo photocontrol of inhibitory brain receptors. **Maleeva G** **O.25.** Three-photon infrared stimulation of endogenous neuroreceptors in vivo. **Sortino R** **0.26.** Deep-phenotype characterization of GRIN1 zebrafish models, a new tool to study GRIN-related disorders. **Locubiche-Serra S** **Llopart N** **O.27.** Comprehensive delineation and precision medicine of GRIN-related neurodevelopmental disorders, a primary disturbance of the NMDA receptor. **Altafaj X** **O.28.** Metabolic characterization of neurodevelopmental disorders involving glutamatergic neurotransmission. **Illescas S**
16.30-17.30	**PLENARY LECTURE** *Prat de la Riba Room* Presented by: X. Altafaj **« Exploring brain cell communication at the single molecule level in health and disease: learning from the NMDA receptor »** Laurent Groc (CNRS/Bordeaux Université)
17.45-19.30h	**Drinks and poster session**

DAY 2: Wednesday, May 29th 2024

9-10.45h	**ORAL SESSIONS** **Session 3A. NEURAL CIRCUITS AND BRAIN PLASTICITY** *Prat de la Riba Room* **Chair: Jordi Bonaventura (Institut de Neurociències, Universitat de Barcelona)** **O.29.** Epigallocatechin-3-gallate pretreatment as a new neuroprotective therapy against polymyxin induced neurotoxicity. **Guzman L** **O.30.** TRESK channel modulates CA3 pyramidal neurons' excitability and hippocampal synaptic plasticity. **Lluís H** **O.31.** Light-dependent cAMP modulation in astrocytes trigger synaptic potentiation, hemodynamic responses and behavioural changes in mice: role in huntington's disease. **Sitjà-Roqueta L** **O.32.** Exploring the dopaminergic effects of S-Ketamine. **Rizzo A** **O.33.** Decoding incidental associations: role of amygdala in sensory preconditioning. **González-Parra JA** **O.34.** Effects of the poly I:C model of schizophrenia on thalamic inhibitory circuits. **Beltran M** **O.35.** Structural plasticity of dendritic spines during long-term synaptic depression. **Rojo-Francàs E** **Session 3B. SENSORY AND MOTOR SYSTEMS** *Pere i Joan Coromines Room* **Chair: Xavier Gasull (Institut de Neurociències, Universitat de Barcelona, IDIBAPS)** **O.36.** Not just a highway: the role of the spinal cord in rats' forelimb skilled function. **López-Santos D** **0.37.** 4-deep brain reconstruction: a 3D-printed mini-brain based on pluripotent stem cells differentiation. **Louail A** **O.38.** Dynamics of noise-induced neurodegeneration and neuroplasticity in the central nervous system. **Giménez-Esbrí V** **O.39.** Transcriptomics and proteomics unravel molecular safeguards of klotho in ALS. **Verdés S** **O.40.** Deciphering the role of extracellular matrix in ALS pathophysiology. **Soares GP** **0.41.** Central and peripheral delivery of ASO 10-27 increases lifespan, improves motor function and preserves excitatory synaptic integrity and neuromuscular junction morphology in a severe mouse model of SMA. **Guillamón P**

	0.42. Superior colliculus as a key player in huntington's disease sensorimotor coordination deficits: from circuits to behaviour. **Küçükerden M**
10.45-12.00	☕ **COFFEE BREAK and POSTERS**
12.00-13.00	**PLENARY LECTURE** *Prat de la Riba Room* Presented by: A.Busquets-Garcia **«Neuron-astrocyte signaling in stress-induced depressive-like states»** Getrudis Perea (Instituto Cajal-CSIC)
13.00-15.00	🍽 **LUNCH and POSTERS**
15.00- 16.45	**ORAL SESSION** **Session 4A. NEURODEGENERATIVE DISEASES II** *Prat de la Riba Room* **Chair: Mireya Plass (Institut d'Investigació Biomèdica de Bellvitge, CIBER-BBN)** **0.43.** Regional and cell-type specific mechanisms of cannabidiol benefit in a model of mitochondrial neuropathy. **Van der Walt G** **O.44.** Alteration of hippocampal CB1R can drive gabaergic dysfunction leading to cognitive decline in Huntington's disease. **Di Franco N** **O.45.** CD200-based cell sorting generates homogeneous subpopulations of transplantable striatal neuroblasts. **Gomis C** **O.46.** Developing an induced pluripotent stem cell (iPSC)-based model to identify the molecular mechanisms governing the neurodegenerative disease Multiple System Atrophy. **Alemany-Ribes M** **0.47.** Increased neurogenesis and behavior performance by in vivo reprogramming. **Zaballa S** **0.48.** Study of cell trafficking for the description of personalized therapies in pediatric movement disorders. **Díaz-Osorio Y** **O.49.** AAV9-mediated expression of secreted klotho reduced several aging-associated phenotypes, improving cognitive capacities and increasing longevity**. Roig-Soriano J** **Session 4B. COGNITION AND MENTAL DISORDERS** *Pere i Joan Coromines Room* **Chair: Albert Giralt (Institut de Neurociències, Universitat de Barcelona)**

	0.50. β-hydroxybutyrate counteracts the deleterious effects of a saturated high-fat diet on synaptic AMPA receptors and cognitive performance. **Fadó R** **0.51.** Sex differences in fear memory processing in mice and humans. **Andero R** **O.52.** Small RNAs are important contributors in the cognitive symptoms associated with schizophrenia. **Galán-Ganga M** **O.53.** Cell type-specific effects of chronic THC exposure on hippocampal plasticity revealed by RNAseq. **Kouchaeknejad A** **0.54.** Understanding the neuro-immune alterations in schizophrenia: the implication of the IKAROS family. **Ballasch I** **O.55.** Astrotactin 1 deficiency results in a novel neurodevelopmental disorder. **Gatnau-Civardi C** **O.56.** Sex-specific changes in miRNA profile in the prefrontal cortex of depressed suicidal subjects. **Miquel-Rio L**
16.45-17.45	**6th RAMON TURRÓ AWARD** honoring the most cited articles in Neurobiology performed in Catalunya published in 1996-98 *Prat de la Riba Room* Presented by: J. Saura **ACCEPTANCE LECTURE** **« The 1996/1998 breaktrough: from Reelin and Cajal-Retzius cells to adult plasticity and Alzheimer´s disease»** Prof. Eduardo Soriano (Institut de Neurociències-Universitat de Barcelona, CIBERNED)
17.45-18.00	**BEST POSTERS AWARDS** at the XIII Symposium *Prat de la Riba room* **CLOSING SPEECH:** Organizing Committee
18.00-20.00	**CLOSING PARTY**

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PLENARY LECTURES

L.1. THE IMPACT OF NEURAL STEM CELL HETEROGENEITY ON NICHE DYNAMICS

FARIÑAS I

[1]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), [2]Departamento de Biología Celular, Biología Funcional y Antropología Física, [3]Instituto de Biotecnología y Biomedicina (BioTecMed), Universidad de Valencia, 46100 Burjassot, Spain.

Most mammalian tissues harbor long-lived stem cells (SCs), which balance their self-renewing proliferation with the production of differentiated progeny to compensate for physiological cell loss. Subsets of SCs can spend long periods in quiescence, but the molecular regulation of this state as well as the reversible transitions between dormancy and activation have started to be understood only recently. Furthermore, subsets of non-cycling SCs in a particular tissue can be found at varying depths of quiescence. While all these cells share a transcriptional program of quiescence, some of them are more prone to engage proliferation upon mitogen stimulation. This state of shallow quiescence has been described in muscle SCs, hematopoietic SCs, and neural SCs (NSCs). Recent refinements in the procedures to isolate NSCs from the adult mouse subependymal zone (SEZ) by fluorescence-activated cell sorting together with deep sequencing and cycling analysis now allows to recognize NSCs in three states: quiescent (q), quiescent-primed (p), and activated (a). Molecularly defined primed-like and activated-like NSCs dividing at different rates can detected in neurospheres growing under mitogenic stimulation suggesting intrinsic properties. The existence and interchangeability of these states suggest finely tuned mechanisms of cycling regulation, the molecular details of which, however, remain unknown. The combination of the molecular knowledge about these states with ways to label quiescent NSCs since mid-gestation by in utero electroporation is helping unravel physical interactions between quiescent NSCs and their niche with impact on cell transitions. Within this new scenario, interactions of NSCs with elements of their niche or systemic elements, such as the immune system, are beginning to be elucidated.

Funding: MICINN (PID, CIBERNED), FEDER, Generalitat Valenciana (Prometeo) and ERC.

NOTES

L.2. EXPLORING BRAIN CELL COMMUNICATION AT THE SINGLE MOLECULE LEVEL IN HEALTH AND DISEASE: LEARNING FROM THE NMDA RECEPTOR

GROC L

University of Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, IINS, UMR 5297, F-33000 Bordeaux, France.

N-Methyl-D-aspartate ionotropic glutamate receptors (NMDARs) play key roles in synaptogenesis, synaptic maturation, long-term plasticity, neuronal network activity, and cognition. Mirroring this wide range of instrumental functions, abnormalities in NMDAR-mediated signaling have been associated with numerous neurological and psychiatric disorders. Thus, identifying the molecular mechanisms underpinning the physiological and pathological contributions of NMDAR has been a major area of investigation. Over the past decades, a large body of literature has flourished, revealing that the physiology of ionotropic glutamate receptors cannot be restricted to fluxing ions, and involves additional facets controlling synaptic transmissions in health and disease. I will discuss newly discovered dimensions of postsynaptic NMDAR signaling supporting neural plasticity and cognition, such as the nanoscale organization of NMDAR complexes, their activity-dependent redistributions, and non-ionotropic signaling capacities. I will further discuss how dysregulations of these processes may directly contribute to NMDAR-dysfunction-related brain diseases.

NOTES

L.3. NEURON-ASTROCYTE SIGNALING IN STRESS-INDUCED DEPRESSIVE-LIKE STATES

PEREA G

Instituto Cajal, CSIC. Av. Doctor Arce, 37. Madrid

Major depressive disorder (MDD) is a severe and debilitating mental illness with a very largesocioeconomic impact worldwide. The neurobiology of this disease has been studied for along time, focused on neuron alterations; however, the underlying etiology is not yet full yunderstood. Astrocytes, a glial cell type, have been shown to play relevant roles modulating synaptic transmission and plasticity,with significant impact on animal behavior. Evidence collected from human samples and animal models have shown that astrocytes shown particular alterations that might contribute to the pathophysiology of MDD. We will discuss recent data showing how stress-induced depression alter astrocytic Ca2+ signalling from medial prefrontal cortex both in vivo and ex-vivo, and also the 5HT-driven cortical synaptic plasticity. We will disucss about the impact of modulating astrocytic Ca2+ signalling for animal behaviour, showing the critical balance between astrocyte Ca2+ signalling and cognitive performance, and the potential role of astrocytes as therapeutic targets in depressive-like states

Supported by grants: PID2019-106579RB-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" by the European Union

NOTES

L.4. THE 1997/1998 BREAKTROUGH: FROM REELIN AND CAJAL-RETZIUS CELLS TO ADULT PLASTICITY AND ALZHEIMER´S DISEASE

SORIANO E

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[2] *Network Center of Biomedical Research in Neurodegenerative Diseases (CIBERNED), 28031 Madrid, Spain.*

In this presentation I will briefly summarize the previous findings that give rise to the two studies to which this Award refers (Del Río et al., 1997, Nature: 385: 70–74; Alcántara et al., J. Neuroscience, 1998, 18: 7779 -7799). The first study demonstrates for the first time a role for Cajal-Retzius cells and Reelin in establishing circuits. The second study represents the first comprehensive analysis of Reelin expression in the brain, highlighting the high expression of this developmental gene in the adult. In addition to delving into these aspects, the two studies opened the doors to analyze the function of Reelin in the adult brain, particularly in synaptic plasticity and adult neurogenesis, demonstrating that Reelin is an enhancer of plasticity. We later proposed that enhancing plasticity could be beneficial to alleviate some neurodegenerative diseases. We demonstrate that Reelin overexpression reduces symptoms and hallmarks in Alzheimer´s Disease mouse models by reducing AB and P-Tau, increasing synaptic potentiation, and enhancing cognitive abilities. These studies demonstrate how a gene originally assigned to neuronal development also controls adult plasticity and may also be useful for neuroprotection against neurodegenerative diseases.

NOTES

SESSION 1A - GLIAL CELLS AND INFLAMATION

O.1. CANNABIDIOL BREAKS THE CHAINS PLACED BY A2AR ON CANNABINOID RECEPTOR 1 IN ISCHEMIC STROKE

Raïch I[1,2], Lillo J[2,3], Rebassa JB[1,2] Rivas-Santiesteban R [2,3], Reyes-Resina I[1,2] , Capó [2,3] , Martínez-Orgado J[4,5] , Franco R [1,2] , Navarro G[1,3,4,*]

[1]Department of Biochemistry and Physiology. School of Pharmacy. University of Barcelona, 08028 Barcelona. Spain.
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[5]Department of Neonatology, Hospital Clínico San Carlos-IdISSC, 28040 Madrid, Spain

In adults stroke is a public Health problem as it is the leading cause of death in women and the second cause of death in men. In addition, in children it is one of the 10 main causes of death. Cannabidiol (CBD) in on of 150 phyticannabinoid compounds that can be obtained from the Cannabis sativa plant. CBD can activate the cannabinoid System without inducing psychoactive effects. In fact, in September 2019, the World Anti-Doping Agency (WADA) recognized the benefits of these compounds for athletes and excluded them from the list of banned substances. CBD is an allosteric modulator of the type 1 cannabinoid receptor (CB1R), the most abundant G protein-coupled receptor (GPCR) in the CNS. It has been repeatedly described that CB1R forms complexes with the adenosine A2AR, another GPCR that is overexpressed under conditions of neuroinflammation, and by activating it to participate in mediation of proliferation and the positive regulation of reactivity of the microglia, causing an impact on neuroinflammation and neurodegeneration.
In our study, we observed that CBD treatment of neuronal and microglial primary cultures, which had previously been in conditions of oxygen and glucose deprivation (GOD), provides an increase in functionality of CB1R as a neuroprotective agent. In parallel, an increase in A2A-CB1 receptor heteromer expression has been detected under GOD conditions, which disappears with pre-treatment with CBD. In the same vein, in animal model of mouse stroke the presence of the A2A-CB1 heteromer increases but if these pups were treated with CBD after surgery and the process of hypoxia the levels of the heteromer were like the controls.
These facts suggest that the A2A-CB1 heteromer may be a good day to reduce this condition of neuroinflammation, with some elements that could increase the neuroprotective function of cannabinoid receptor.

Proyecto SAF del Ministerio de España (SAF2017-84117-R)

NOTES

O.2. PHENOTYPIC CHARACTERIZATION OF LIPID DROPLET-ACCUMULATING MICROGLIA AFTER CEREBRAL ISCHEMIA

Pedragosa J[1], Chaparro-Cabanillas N[1,6], Honores M[2], Serra-Moral J[1,5], Arbaizar M[1,2], Pol A[2,3,4], Planas AM[1,2]

[1]Department of Neuroscience and Experimental Therapeutics, Institut d'Investigacions Biomèdiques de Barcelona (IIBB), Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain.
[2]Fundació Recerca Clínic Barcelona-IDIBAPS, Barcelona.
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[5]Universitat Pompeu Fabra
[6] Universitat de Barcelona

Background: Lipid droplets (LDs) are cytosolic lipid storage organelles generated in response to metabolic demands or inflammatory/immune challenges. Following cerebral ischemia, microglia of mice and stroke patients show LD biogenesis, with induction of LDs-associated molecules like perilipin-2 (Plin2). Plin2 is as a structural protein of LDs that regulates LD stability and control lipolysis.
Objective: We aim to investigate the dynamics of LD accumulation in microglia over time after cerebral ischemia and establish a protocol to characterize and measure microglia phenotypic changes and LD storage.
Methods: Transient cerebral ischemia was induced in Tmem119-creERT mice expressing a fluorescent protein (tdTomato) in microglia only. The brain lesion was confirmed by T2w-MRI. Immunofluorescence and confocal microscopy were carried out to characterize Plin2 accumulation in microglia and other phenotypic alterations. Morphometric changes in microglia were assessed using image analysis tools.
Results: Microglia presents a gradual accumulation of Plin2, indicative of LD biogenesis, progressing from the periphery to the ischemic core, and show morphological changes in perimeter, circularity and solidity 4 days post-stroke. At this time point, microglia show signs of phagocytic activity (CD68+) and they proliferate (Ki67+). After 15 days, microglia and astrocytes form a dense glial scar surrounding the ischemic core, while the glial cell proliferative activity is negligible and the presence of microglia containing LDs is strongly reduced.
Conclusion: Microglia form a barrier around the lesion starting 4 days after stroke with increasing cell density at day 15. Microgliosis occurs due to the proliferation of microglial cells that contain LDs. The strong reduction of LDs in microglia 15 days after stroke may be due to consumption of stored lipids during the highly demanding period of microglia clearance of damaged tissue and cell division.

Study supported by a Grant from ' Fundació "La Caixa" (Ref. 2022-HR23-00560).

NOTES

O.3. Intravenous Gene Therapy Provides Long-Term Correction of Cerebral White Matter Swelling and Locomotor Deficiencies in a Mouse Model of Megalencephalic Leukoencephalopathy with Subcortical Cysts

Brao A[1,2], Sánchez Á[1,2] , Rodríguez I[1,2] , Prat E[3,4] , Nunes V[3,4] , Chillón M[1,2,5] , Estévez R[3,4] Bosch A[1,2,6]

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC, also known as Van der Knaap disease) is an ultrarare, infantile onset leukodystrophy characterized by cerebral white matter edema for which there is no available treatment. Most patients bear loss-of-function mutations in MLC1 (~75%) and develop early-onset macrocephaly, cerebellar ataxia, spasticity and muscle stiffness, progressive motor deterioration, epileptic seizures, and mild cognitive decline with or without autism (including learning difficulties and speech deterioration). MLC1 encodes an integral membrane protein which is specific to the astrocytic lineage and, while its function is yet to be fully elucidated, its deficiency is associated with the dysregulation of brain water and ion homeostasis mechanisms, leading to the progressive vacuolation of myelin sheaths and astrocytic endfeet.
To design a gene therapy for MLC, we administered an adeno-associated viral vector (AAV) capable of crossing the murine blood-brain barrier containing the human MLC1 cDNA under the control of a human astrocyte-specific promoter, gfa2, to 10-month-old Mlc1-/- mouse, several months after the onset of white matter vacuolation. Despite the late therapeutic approach, in vivo magnetic resonance imaging (MRI) revealed a complete normalization of signal abnormalities associated with brain water accumulation (e.g., T2 or apparent diffusion coefficient), as confirmed by subsequent histopathological analyses. Furthermore, we detected long-term astrocyte-driven expression of MLC1 as later as 1 year after viral vector administration in all brain areas analysed. Importantly, our therapy provided a steady, but prolonged rescue of motor deficits in this mouse model. All in all, our results demonstrate the longstanding effectiveness of this non-invasive gene therapy, confirm the wide window for therapeutic intervention, and provide new insights into the pathophysiology of MLC.

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NOTES

O.4. CANNABIDIOL EXTENDS LIFESPAN AND IMPROVES CLINICAL SIGNS IN A MOUSE MODEL OF LEIGH SYNDROME

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Mutations in mitochondrial energy-producing genes lead to a heterogeneous group of untreatable disorders known as primary mitochondrial diseases (MD). Leigh syndrome (LS) is the most common pediatric MD and is characterized by progressive neuromuscular affectation and premature death. Here, we show that daily cannabidiol (CBD) administration significantly extends lifespan and ameliorates pathology in two LS mouse models. These effects were correlated with a decrease in both astrogliosis and microgliosis, as well as a normalization of an altered microglial morphology observed in an affected brain area of a LS mouse model. Moreover, we found that CBD reduces oxidative stress and ameliorates the phenotype of LS patient-derived fibroblasts. Taken together, our results provide the first evidence for CBD as a potential treatment for LS.

Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Universitat Autònoma de Barcelona (UAB), Ministerio de Economía y Competitividad, Consejo Europeo de Investigación (ERC)

NOTES

O.5. ASTROCYTIC RTP801 IS INVOLVED IN NEURODEGENERATION AND NEUROINFLAMMATION IN ALZHEIMER DISEASE.

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Neuroinflammation is a key player in many neurodegenerative diseases, including Alzheimer's disease (AD). In this process, astrocytes and microglia are the main cells involved, releasing cytokines, chemokines, NO, and ROS. Neuroinflammation has devastating consequences, as it leads to neuronal death, synaptic dysfunction and inhibition of neurogenesis. The protein RTP801 is involved in neuroinflammation, which levels are higher in the hippocampus of AD patients. Such increased levels of neuronal hippocampal RTP801 correlated with the Thal and Braak stages of the disease, and astrogliosis. Moreover, silencing RTP801 in hippocampal neurons prevented cognitive impairment and neuroinflammation in the 5xFAD mouse model of AD. Studies of neurons alone failed to understand how circuit plasticity is established and maintained. Astrocytes play an essential role in neuron trophic support, neurotransmitters uptake and recycling and synapse formation. All these functions are hampered when the astrocytes become reactive.
This study aims to assess whether astrocytic RTP801 affects memory and neuroinflammation in the 5xFAD mouse model. RTP801 was knocked-down specifically in astrocytes. We performed behavior tests to test spatial memory and anxiety-like behaviors. Silencing astrocytic RTP801 (miRTP801) in 5xFAD mice recovers the anxiety-like phenotype at the Plus Maze test and improved the spatial learning and memory evaluated by the Morris water maze. Mice were subjected to MRS (Magnetic resonance spectroscopy) and resting-state functional connectivity. Thus, brain metabolites were detected and quantified. Silencing RTP801 in hippocampal astrocytes in the 5xFAD mice prevented the loss of the GABAergic signaling and overall increases the connectivity. Furthermore, silencing RTP801 in 5xFAD hippocampal astrocytes reduced microgliosis and astrogliosis, as well as the levels of some the inflammasome components effectors such as NLRP3, ASC, and pro-caspase-1 compared to miCT injected 5xFAD. Hence, we conclude that astrocytic RTP801 is contributing to cognitive impairment by affecting GABAergic signaling and the inflammatory response in the pathogenic context of AD.

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NOTES

O.6. THE GUT-BRAIN AXIS IN A NOVEL HUMANIZED TRANSGENIC MOUSE MODEL FOR PARKINSON´S DISEASE AND BRAIN AGING

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Accumulating evidence indicate that alterations in the gastrointestinal (GI) function and the gut microbiota represent a risk factor for Parkinson's disease (PD). Changes in the gut-brain axis can affect both the enteric and central nervous systems, which might have implications in understanding disease pathophysiology and for the development of disease modifying therapeutic strategies.
To clarify how the gut-brain axis is involved in disease pathogenesis and/or in modulating the manifestation of PD symptoms, we used a new transgenic neuromelanin-producing mouse model (tgNM) that mimics the age-dependent accumulation and brain-wide distribution of neuromelanin occurring in humans.
These animals exhibit gastrointestinal dysfunction (i.e. altered fecal output, gut permeability) in the prodromal phase before the appearance of dopaminergic dysfunction. These functional alterations correlated with an altered composition of the fecal microbiome and metabolome, as well as increased fecal and intestinal inflammation markers. Next, we assessed if modulation of the gut microbiota could affect the manifestation of both motor and non-motor symptoms in tgNM mice. Thus, we fed the animals with a high fat diet (HFD) and evaluated the worsening of the peripheral symptomatology and brain pathology observed in tgNM mice.
Our results indicate that modelling human brain pigmentation in mice is sufficient to induce prodromal gastrointestinal dysfunction and that the newly generated neuromelanin-producing mouse model represents a valuable tool to test disease-modifying strategies for PD based on the modulation of the gut-brain axis.

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NOTES

O.7. ELUCIDATING THE ROLE OF ATF3 IN THE NEUROPATHOLOGY OF A MOUSE MODEL OF LEIGH SYNDROME

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Mitochondrial energy-generating machinery mutations cause mitochondrial disease (MD), a diverse group of orphan neuromyopathies. Leigh Syndrome (LS) is the most common pediatric MD, leading to severe encephalopathy and premature death. Albeit clinically heterogenous, LS is characterized by progressive neuronal damage, which is anatomically restricted to brainstem and basal ganglia, revealing that not all neurons are equally susceptible to mitochondrial dysfunction.
We have previously demonstrated that GABAergic and glutamatergic neurons are vulnerable to the disease in mice lacking the Ndufs4 subunit (Ndufs4KO), a well-characterized model of LS that recapitulates the human disease. However, the underlying mechanisms leading to neuronal demise remain unknown, underscoring the need to develop novel treatments, as it remains an incurable pathology.
To dissect the mechanisms affected in glutamatergic neurons, we combined mouse genetics with cell-type specific transcriptomics. RNASeq results showed an upregulation of the activating transcription factor 3 (Atf3) in glutamatergic neurons in the vestibular nuclei of Ndufs4KO, an area severely affected by the disease. Remarkably, induction of this transcription factor is also observed in both NDUF4-deficient human brain organoids and brains from Leigh Syndrome patients, suggesting that this is a conserved response to mitochondrial dysfunction.
To dissect the contribution of ATF3 to the pathology, we generated and characterized a Ndufs4KO mouse line lacking ATF3. Our results show that deletion of Atf3 rescues synaptic loss and attenuates microglial reactivity in glutamatergic brain regions of Ndufs4KO mice, coincident with a partial restoration of breathing impairments without any effect on lifespan.
Overall, our results highlight a role for ATF3 in the development of microglial reactivity and neuronal synaptic loss in the context of mitochondrial dysfunction. These results offer a potential therapeutic approach for the neurodegenerative processes underlying MD.

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NOTES

SESSION 1B - NEURODEVELOPMENT AND RELATED DISEASES

O.8. CXCL14 IS A KEY CHEMOKINE INVOLVED IN AXON GUIDANCE OF PIONEER AXONS OF THE STATOACOUSTIC GANGLION

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Bipolar neurons of the inner ear are located in the statoacoustic (SAG) ganglion and extend to hair cells and the second order neurons of the brainstem. We have recently shown that SAG organization relies on pioneer neurons (Bañon and Alsina, 2023). Here we show, by in vivo imaging of the pioneer axons in the intact zebrafish, how the pioneer axons grow, branch, refine and target newborn hair cells of the otic vesicle. Moreover, we reveal that the pioneer axons are used as scaffolds for newly delaminated neuroblasts to migrate and establish the posterior lobe of the SAG. But how pioneer axons reach precisely the newborn hair cells? We have identified the chemokine Cxcl14, expressed in otic hair cells from 24hpf onwards, as a pioneer axon guidance and/or fasciculation cue. A lack of Cxcl14 due to a CRISPR/Cas9 KO results in defasciculation of pioneer axons, incorrect targeting to hair cells and malformation of the posterior lobe. We also find defects on SAG and lateral line axons entering the hindbrain in mutant embryos, as Cxcl14 is also expressed in two spots adjacent to the hindbrain that correspond to the hindbrain entry points. SAG development occurs in close contact with anterior and posterior lateral line ganglia, which progressively become separated from SAG and send axons towards hair cells of the neuromasts. Interestingly, we do not find Cxcl14 expression in hair cells of the neuromasts, suggesting that Cxcl14 plays a key role in regulating the correct targeting of SAG towards otic hair cells but not hair cells of the neuromasts. Altogether, our work shows for the first time how pioneer axons extend and the role of the chemokine Cxcl14 in guiding the pioneer axons and establishing a correct inner ear circuitry.

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NOTES

O.9. ASTROTACTIN 1 DEFICIENCY RESULTS IN A NOVEL NEURODEVELOPMENTAL DISORDER

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Astrotactin 1 (Astn1) is a glycoprotein expressed during neurodevelopment. It is a neuronal adhesion molecule required for normal migration of young postmitotic neuroblasts along glial fibers. There are no pathologies associated to any variants in ASTN1, but some linkage studies suggest an association between deletions and duplications in ASNT1 and neurodevelopmental disorders such as ASD, ADHD, and OCD.

In this work we report a patient presenting with neurodevelopmental delay, focal epilepsy and autism spectrum disorder characteristics. Genetic analysis revealed the homozygous variant ASTN1 c.1523+1G>T. Since it is the first ever patient diagnosed with a neurological phenotype due to mutations in ASTN1, we performed a whole personalized study of the mutation and its impact in neuronal homeostasis.

We first investigated the effect of the mutation in ASTN1 expression in patient derived fibroblasts, upon confirmation of a detectable expression of the gene in this cell type. We found that the mutation yielded to a severe reduction in the mRNA levels, probably through the activation of the Nonsense-mediated mRNA decay (NMD) pathway. Interestingly, it was associated with an increase in the expression of ASTN2, which interact for glial-guided neuronal migration. Moving forward to the analysis in neuronal

cells, we inhibited the expression of ASTN1 by means of shRNA, observing abnormalities in neuronal homeostasis, reinforcing the importance of this gene in neurodevelopment. The associated alterations in neurotransmitter receptors shape the avenue for future personalized treatments.

In summary, this ongoing work is the first report of a patient bearing mutations in ASTN1, confirming its involvement in neurodevelopment and setting the first stone on the description of the natural history of the disease. The personalized study of the pathophysiology gives way to potential therapeutic strategies for this and other neurodevelopmental disorders.

NOTES

O.10. 4D-STUDY OF NEURONAL CELL LINEAGES AND THE GENERATION OF NEURONAL DIVERSITY IN THE ZEBRAFISH HINDBRAIN

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The comprehension of how the brain is built and its cell diversity established during embryogenesis is key to understand its function. Brain development requires an exquisite balance between proliferation and differentiation of the distinct progenitor populations to generate the correct number of neurons, at the right place and time. This process can be assessed by studying cell lineages and the implicated molecular pathways.
The hindbrain, the most evolutionary conserved brain vesicle among vertebrates, is responsible for vital roles, such as respiration, circulation or motor coordination. It is also an excellent model for studying how cell lineages contribute to organ growth in space and time, since it undergoes profound morphogenetic changes as development proceeds.
We perform high-resolution 4D live imaging of zebrafish embryos using lineage tracing to unravel how specific proneural gene-expressing progenitor populations of the hindbrain contribute to its growth. With that purpose, we have developed a custom-made algorithm to detect nuclear centers in 3D, in a highly compact tissue, where nuclear fluorescent signals from adjacent nuclei highly overlap with one another. Then, we have used already-available software to track these centers in time (4D) and thus determine the mode of division of cells. In parallel, we have used the multicolor clonal analysis technique Zebrabow to assess the stochastic or deterministic nature in the neuronal output (number of neurons) of individual progenitors.
Finally, we have examined the role of the Notch pathway in the maintenance of the balance between proliferation and differentiation, and how it regulates the neuronal output of proneural gene-expressing populations.
The information in the mode of division of the progenitors, the number of neurons they produce and the molecular pathways involved will allow us to understand how the hindbrain is formed.

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NOTES

O.11. THE HIDDEN SIDE OF NCAM FAMILY: NCAM2, A KEY CYTOSKELETON ORGANIZATION MOLECULE REGULATING NEURONAL DIFFERENTATION AND SYNAPTIC FORMATION IN BRAIN DEVELOPMENT

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Neural Cell Adhesion Molecule 2 (NCAM2) is a cell adhesion molecule which is expressed in the brain. While its role has been extensively investigated in the olfactory system, little was known about its functions in hippocampus and cortex. Our research aimed to increase understanding of the molecular mechanisms related with NCAM2 and its involvement in several aspects of brain development.

We found that NCAM2 is a crucial protein for proper neuronal morphogenesis and cortex formation. Specifically, NCAM2 depletion in vitro has dramatic effects on neurite development and polarization. Furthermore, we demonstrated the role of NCAM2 in cortical neuronal migration. Biochemical experiments provided evidence that NCAM2 exerts the above functions by modulating microtubule dynamics and by interacting with the microtubule-associated protein MAP2 and certain members of the 14-3-3 protein family.

In our studies, we characterized the interactome of NCAM2 during postnatal cortical development, 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 functions, such as dendritic tree development and synapse formation.

In the adult brain, we observed that NCAM2 is important for the proper synaptic plasticity. Our results indicate that NCAM2 contributes to synapse formation and stability; and that physiological levels NCAM2 are necessary for synapse maintenance in the adult nervous system.

Our studies unveiled the importance of NCAM2 in neuronal differentiation and synapse formation, with some of these functions being related to the ability to NCAM2's to modulate the cytoskeleton. These findings shed light on novel functions of NCAM2 in neural development through a previously unknown NCAM2/cytoskeletal protein complex. Our results described mechanisms that could help explain some pathologies linked to NCAM2, which exhibit a complex phenotype including altered brain development and synaptogenesis.

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NOTES

O.12. THE ROLE OF THE RNA-BINDING PROTEIN STAUFEN 2 DURING NEUROGENESIS

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Neurogenesis is a crucial process in which new neurons are formed in the brain cortex in the embryo. Recent studies indicate that post-transcriptional regulation of gene expression by RNA binding proteins (RBPs) is a critical step in fine-tuning neurogenesis and mediating cortical development. However, little is known about the identity and role of these RNA regulatory networks during this process. Staufen 2 (STAU2) is a double-stranded RBP that has been implicated in the asymmetric distribution of mRNAs in radial glial cells (RGCs), thereby dysregulating the balance between neural stem cell maintenance and differentiation during mouse brain development. To unravel the RNA regulatory networks controlled by STAU2 at different stages of human neurogenesis, we performed single-cell RNA-seq (scRNA-seq) on different neurogenic populations derived from STAU2 KO and control human induced pluripotent stem cells (hiPSCs). The samples were sequenced at multiple timepoints, reflecting the transition from hiPSCs to mature neuronal and glial cell types. Differential Expression analysis suggests that STAU2 may play a role in early neurogenesis where the maximum number of DEGs are detected. Interestingly, the identified dysregulated transcripts are related with neuronal differentiation and maturation processes which are not detected in control cell line at this stage. In Immunohistochemical and qPCR analyses of differentiated neuroepithelial cell cultures, we observed indeed a significant enrichment of Map2+ and Tuj1+ neuronal populations compared to the control cell line. Taken together, these data provide new insights into the functional role of Stau2 at the neuroepithelial stage and confirm our Stau2 knock-down cell line as a valid model to further identify and analyze Stau2 protein and RNA regulatory networks during human neurogenesis.

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NOTES

O.13. KETOGENIC DIET AS A POTENTIAL TREATMENT FOR SPATA5-RELATED ENCEPHALOPATHY: THE POWER OF MITOCHONDRIAL MODULATION IN THE TREATMENT OF NEURODEVELOPMENTAL DISEASES

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Mutations in SPATA5, an ATP-dependent chaperone that plays an essential role in the cytoplasmic maturation steps of ribosomal particles, can lead to childhood-onset encephalopathy. Up to date, only 40 patients presenting mutations in this gene have been described, and the molecular causes of the disease, and therefore potential therapies, remain unclear.
In this work, we have analyzed the mitochondrial phenotype associated to SPATA5 deficiency, building on previous works reporting alterations regarding mitochondrial homeostasis in SPATA5-deficient neurons. In vitro analysis of the mitochondrial function in patients' primary fibroblasts revealed an alteration in bioenergetics by means of a decrease in ATP production and increase in oxidative stress, detected by bioluminescence and flow cytometry, respectively. In vivo time-lapse confocal microscopy revealed alterations in mitochondrial dynamics as well, as patients' fibroblasts showed an aberrant mitochondrial network with decreased motility, translated into mitochondrial fusion-fission ratio alterations.
Since the epileptic phenotype associated with SPATA5 deficiency is refractory to most pharmacological treatments, we investigated the impact in mitochondrial homeostasis of ketogenic diet (KD), a neurometabolic approach to the management of refractory seizures. Treatment of the fibroblasts with a KD-mimicking media recovered the mitochondrial dysfunction in the patients' fibroblasts, both in bioenergetics and dynamics behavior. Enlightened by these results, four patients with refractory epilepsy were treated with KD, resulting in seizure reduction in three of them, along with improvements in attention, behavior, and motor skills.
Our results highlight the importance of understanding the pathophysiology of SPATA5-related encephalopathy (SPATA5-RE) to find new treatment options. Together with the in vitro characterization, this work is the largest description of the natural history of this rare neuropediatric disease, reviewing the 40 published patients and 11 new ones. The improvement of mitochondrial homeostasis by KD could explain the clinical benefits in seizure control, constituting it as a personalized treatment option for SPATA5-RE patients.

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NOTES

O.14. NOVEL STRATEGIES TO DECRYPT THE COMPLEXITY OF GROWTH CONE RESPONSES: FROM SIGNAL INTEGRATION TO MOTILE RESPONSES

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The complex connectivity of the nervous system arises during development as a result of sequential mechanisms of neuronal migration, axon guidance and synaptogenesis that generate standardized patterns of innervation. During guidance, axons navigate the extracellular milieu responding either positively or negatively to guidance cues, molecular tags that often have a dual role and act both as chemoattractants and chemorepellents. The response of a particular axon is given by the unique repertoire of guidance receptors and intracellular factors expressed by the growth cone, the pivot point of axon guidance.
Guidance cues activate signaling cascades that modify second messenger concentration, especially cAMP, cGMP and calcium, and lead to intracellular rearrangements of the cytoskeleton and changes in the dynamics of exocytosis and endocytosis within the growth cone, inducing its turning towards or away from the cue and the elongation or retraction of the axon. Second messengers are critical regulators of a myriad of cellular functions. Beyond a simple variation of their concentration, their signals are coded to activate specifically one of their downstream pathways. The exquisite directionality of the growth cone response further requires the confinement of cellular effectors, such as membrane remodeling and cytoskeletal reorganization.
Conventional methods to investigate axon guidance fail to identify the intricacies axon responses. We recently engineered SponGee and SpiCee, genetically-encoded buffers for cGMP and calcium with cellular and subcellular specificity. Using these tools, we demonstrated that ephrin-A5 and Slit1, two axon repellents, induce opposed spatially-segregated second messenger signals. These signals control particular morphological changes in the growth cone and define distinct axon navigation responses in vivo, indicating that what we understand by axon repulsion is far more complex than we anticipated.
Similarly, we are developing new tools to interfere with cellular effectors, to understand the mechanisms that confine growth cone responses and precisely steer the axon.

Agence Nationale de la Recherche Française ANR, Agència Catalana de Gestió d'ajuts Universitaris i de Recerca AGAUR.

NOTES

SESSION 2A - NEURODEGENERATIVE DISEASES I

O.15. ROLE OF NR2A AND NR2B ON THE NEUROPROTECTIVE EFFECTS OF CB1R IN ALZHEIMER'S DISEASE.

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Despite extensive research, effective treatment for Alzheimer's disease (AD) remains elusive. Emerging evidence suggests that the endocannabinoid system and glutamatergic neurotransmission play important roles in AD pathophysiology. This project aims to elucidate the involvement of cannabinoid type 1 receptor (CB1R) on regulating the cytotoxic effects induced by N-methyl-D-aspartate receptors (NMDARs) in in AD.
On the one hand, CB1R modulates neurotransmitter release and synaptic plasticity. Preclinical studies demonstrate that CB1R activation ameliorates cognitive deficits, reduces neuroinflammation, and promotes neuroprotection in AD models. On the other hand, the synaptic receptor NR2A contribute to synaptic integrity and memory development. It has been observed that Aβ specifically activate extra synaptic NMDARs exacerbating synaptic dysfunction and neurodegeneration in AD brains. Also, the extra synaptic NMDAR containing NR2B subunit induces neurotoxicity and excitotoxicity.
Emerging evidence suggests a complex interplay between CB1R and NMDARs. At the functional level, it has been observed a negative cross-talk induced by CB1R in the NR2B signaling in transfected HEK-293T cells. It has also been demonstrated a potentiation of the endocannabinoid signaling by the presence of NMDAR containing NR2A subunit in comparison with NR2B in transfected HEK-293T and cortical neurons. This effect is also observed in the AD 5XFAD mice model. However, the activation of the NMDAR containing NR2B subunit in the presence of the Aβ peptide downregulates the CB1 receptor functionality. Finally, in 5XFAD mice model, it has been demonstrated a decrease in p-Tau mobilization by CB1R activation in neurons KO for the NR2B subunit.
Taken together, these results point that future research should focus on elucidating the molecular mechanisms underlying CB1-NMDAR interactions and evaluating new therapies in AD patients.

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NOTES

O.16. CELL-TYPE SPECIFIC HIPPOCAMPAL ALTERATIONS ARE ASSOCIATED WITH MEMORY DEFICITS IN NOVEL ALZHEIMER'S DISEASE TRANSGENIC MICE

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Excitatory/inhibitory neurotransmission imbalance in memory neural circuits has been proposed to underlie memory deficits in Alzheimer's disease (AD), but the cellular mechanisms underlying specific changes in excitatory and inhibitory hippocampal neurons remain largely unclear. Here, we report differential deleterious effects of amyloid-β and tau on hippocampal-dependent memory and synaptic plasticity associated with cell type-specific pathology in novel double APP/Tau transgenic mice expressing familial AD-linked mutant human amyloid precursor protein (APP) and microtubule-associated protein tau (MAPT) genes in excitatory neurons. Histopathological analyses reveal that amyloid- (Aβ) and phosphorylated Tau are acumulated in CaMKIIα-positive excitatory neurons in the hippocampus of APP/Tau transgenic mice. Tau and APP/Tau mice show spatial learning and memory deficits, which are associated with accumulation of phosphorylated Tau and reduced levels of synaptic proteins at excitatory synapses in the hippocampus. Importantly, synaptic dysfunction coincides with Tau accumulation at excitatory synapses, as revealed by biochemical and expansion microscopy analyses. Interestingly, APP/Tau mice show region-specific reduction and altered morphology of hippocampal inhibitory parvalbumin (Pvalb)-expressing interneurons. Likewise, cell-type specific mRNA-seq revealed transcriptional alterations related to synaptic plasticity, axon maintenance and cell-cell adhesion in Pvalb interneurons. In summary, APP/Tau mice show cell-type specific pathological alterations in memory neural circuits making this model a useful tool for studying the molecular mechanisms underlying selective cellular vulnerability in AD.

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NOTES

O.17. EVALUATION OF THE FUNCTIONAL ACTIVITY IN SYNAPTIC GENES RELATED TO POLYGENIC RISK FOR ALZHEIMER'S DISEASE USING A MASSIVELY PARALLEL REPORTER ASSAY

Perlaza D[1], Lidón L[1,2] , Cervantes-González A[1,2] , Serrano-Requena S[1], Valle-Tamayo N[1,2] , Sánchez-Aced E[1,2] , Álvarez-Sánchez E[1], Aumatell-Escabias J[1], Muñoz L[1,2] , Sirisi-Dolcet S[1,2] , Dols-Icardo O[1,2] , Lleó A[1,2] , Belbin O [1,2]

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Alzheimer's disease (AD) is a synaptopathy. In 2021, we generated a synaptic Polygenic Risk Score (PRS) comprising 8 variants within 6 synaptic genes (APOE, PICALM, BIN1, PTK2B, DLG2, and MINK1), that predicted AD with 72% accuracy in two neuropathological cohorts, supporting the hypothesis that genetic variants that regulate an individual's vulnerability to AD-related synapse degeneration could be used to identify individuals at-risk for AD prior to the appearance of clinical symptoms. This study aims to determine whether the constituent variants of the linkage disequilibrium (LD) blocks represented by the PRS have regulatory activity in vitro.
We cloned an oligonucleotide library of 137 putative regulatory variants, each represented by 5 barcodes per allele, into the pMPRA1 vector. These plasmids were transfected into HEK293 cells (n=5), and DNA/RNA were extracted and sequenced on an Illumina MiSeq. Using the mpra package in R, we normalized the tag counts to a common size of 10 million reads and computed paired log ratios of RNA/DNA counts for each barcode. We used weighted linear models for testing differential activity of minor versus major alleles of each SNP using the mpralm function, adjusting for multiple testing with the Benjamini-Hochberg method.
We acquired approximately 15 million reads of DNA and RNA from 5 independent experiments. 35 out of 137 SNPs tested exhibited differential activity between alleles (adj. $p<0.05$). Three SNPs with regulatory activity were in the PRS (BIN1: rs17014923 and rs35114168; DLG2: rs286043), while the remaining 32 were captured on LD blocks within the synaptic PRS.
All LD captured by the synaptic PRS contained SNPs impacting regulatory activity, supporting a potential mechanism where changes in expression of these loci encoding synaptic proteins could modify cumulative AD risk. Further studies elucidating the precise mechanism involved in this regulatory activity at the synapse could guide future therapeutic strategies for AD.

Study funded by: PI21/00063: Translational study of polygenic risk and protein biomarkers for the early detection and monitoring of synapse vulnerability in Alzheimer's disease

NOTES

O.18. SINGLE-CELL TRANSCRIPTOMICS ANALYSES UNVEIL POST-TRANSCRIPTIONAL REGULATION ALTERATIONS IN IPSC-DERIVED NEURON AND GLIA CELLS FROM ALZHEIMER'S DISEASE PATIENTS

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Alzheimer's disease (AD) is the most common cause of neurodegeneration and dementia, but its pathogenesis remains poorly understood. Post-transcriptional regulation, e.g. via RNA-binding proteins (RBPs) or alternative poly-adenylation (APA) has been shown to be implicated in AD among other mechanism. However, whether the corresponding alterations play a causative role or rather represent consequential symptoms has not yet been elucidated.
To address this question, we have investigated the transcriptional profile of iPSC-derived neuron and glia cells from sporadic AD patients and healthy controls across 70 days of in vitro differentiation.
scRNA-seq analysis identified differential gene expression changes time-point and cell type specific between AD patients and healthy controls. Several of those have been previously associated with AD, such as NNAT upregulation in cortical progenitors and excitatory neurons and downregulation of C1orf61 in astroglia progenitors. Others are RBPs, such as NOVA1 and NCL that are upregulated in excitatory neurons and neural precursor cells respectively. We have also studied the differential 3' UTR usage with SCALPEL, a new tool that we have developed to quantify the abundance of transcripts isoforms generated by APA. SCALPEL analysis revealed that several genes previously implicated in AD pathogenesis display changes in APA between AD and control samples including ELAVL4 in inhibitory neurons.
Taken together, our results show that iPSC-derived neurons and astrocytes from sporadic AD patients present RBP alterations and RNA processing defects that could be studied in the future as potential new therapeutic targets before the onset of the disease.

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NOTES

O.19. PATHOLOGICAL INTERPLAY BETWEEN ALPHA-SYNUCLEIN AND NEUROMELANIN ACCELERATES PARKINSON'S DISEASE PATHOLOGY IN MELANIZED RODENTS

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Parkinson's disease (PD) is characterized by a preferential loss of neurons containing the pigment neuromelanin (NM), especially dopaminergic neurons of the substantia nigra (SN), and the presence in affected neurons of alpha-synuclein (αSyn)-containing cytoplasmic aggregates termed Lewy bodies (LB). While αSyn aggregation is considered a central pathogenic event in PD, mechanisms and significance of LB formation remain unknown. It has been reported that αSyn redistributes to the lipid component of NM at early PD stages and is entrapped within NM granules extracted from PD, but not control, brains. However, it has not yet been possible to experimentally assess in vivo a potential pathological interaction between αSyn and NM because, in contrast to humans, NM is absent in common experimental animals.
We have developed the first rodent model of human-like NM production based on the viral vector-mediated nigral expression of melanin-producing enzyme tyrosinase (AAV-TYR). Age dependent NM accumulation in TYR-injected rats triggers a progressive PD-like phenotype.
Here we aim to assess the potential crosstalk between αSyn and NM by combining αSyn overexpression with TYR-induced NM production in rodents.
Human αSyn overexpression in TYR-expressing rats resulted in an increase of intracellular NM levels, a more sustained formation of LB-like inclusions, an exacerbated presence of αSyn oligomeric species and an enhanced nigrostriatal degeneration. Increased NM levels in αSyn/TYR co-expressing animals was linked to a permeabilization of dopamine-containing synaptic vesicles and subsequent release of dopamine to the cytosol where it oxidizes into NM, as shown by decreased dopaminergic vesicular uptake in these animals by mass spectrometry.
Overall, our results indicate that increased levels of αSyn, as it occurs in PD patients, may accelerate age-dependent NM accumulation and enhance NM-linked PD pathology. Elucidating the pathogenic interplay between αSyn and NM will thus be crucial for understanding PD etiopathogenesis and for the development of novel disease-modifying therapeutic strategies.

Study funded by: Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

NOTES

O.20. DECIPHERING SEROTONERGIC SYNAPTIC ALTERATIONS IN SYNUCLEIONOPATHY AND DEPRESSION COPATHOLOGY MOUSE MODEL

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Anxiety and depression affect 35-50% of patients with Parkinson's disease (PD). In the serotonin (5-HT) system alpha-synuclein (a-Syn) aggregates are present and functional changes are involved in the PD prodromal phase and contribute to non-motor symptoms. Using a mouse model of depression and synucleinopathy in raphe 5-HT neurons, we aim to evaluate: 1- behavioral phenotype, 2- synaptic changes in prefrontal cortex and striatum, and 3- possible sex-based differences.

Recombinant adeno-associated viral vectors (AAV2/5) encoding wild-type human a-Syn (h-a-Syn) were infused in raphe nuclei of male/female mice. The behavioral phenotype was assessed at 4- and 8-weeks post-infusion. Confocal microscopy was used to examine cortical and striatal brain sections immunostained for synapse-related proteins. Fluorescence activated synaptosome sorting (FASS) was performed to obtain purified and enriched cortical and striatal synaptosomes from VGLUT1-Venus mice infused with AAV2/5, in which 5-HT markers were evaluated in association with both excitatory and inhibitory partners. Statistical significance was determined by t-tests or one-way ANOVA.

Overexpression of raphe h-a-Syn induced a sex-dependent depressive-like phenotype and anxiety-like behavior in female, which was absent in male mice. We found a progressive increase in axonal swellings in the striatum and prefrontal cortex, where h-a-Syn colocalized with serotonin transporter (SERT). In parallel, we measured the decrease in microtubule-associated protein 2 (MAP2) and found increases in synaptic vesicle 2A (SV2A) and synaptophysin (SYP) proteins in the same brain areas of 5-HT projection.

Overexpression of h-a-Syn in the mouse 5-HT system induces a sex-specific behavioral phenotype and synaptic pathology in interconnected brain areas.

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NOTES

O.21. THE UNCHARTED NON-CODING LAYER OF THE HUMAN TRANSCRIPTOME REVEALS POTENTIAL NEW PLAYERS IN BRAIN (PATHO)PHYSIOLOGY

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Accurate mapping of genes and transcripts is crucial for unraveling the biological information embedded in genome sequences. GENCODE, as an international consortium aiming at delineating comprehensive annotations for human and mouse genomes, has become a reference for thousands of studies in Biology and Biomedicine. However, despite their involvement in various physiological processes and a growing number of diseases, long non-coding RNAs (lncRNAs) remain inadequately annotated, hindering a thorough understanding of their cellular roles. To address this gap, the CapTrap-CLS strategy was employed to enrich 5'-capped, full-length RNAs from unexplored genomic regions across diverse tissues and developmental stages. This enabled the elucidation of thousands of previously uncharacterized transcripts, with brain exhibiting a particularly high proportion of such novel loci. Thus, to illustrate the relevance of using a comprehensive annotation in the study of neurodegenerative diseases, we re-quantified publicly available RNA-Seq samples spanning different brain areas and disorders, employing the GENCODEv42 annotation enhanced with the novel transcripts detected. Focusing on Alzheimer's disease as a case study, our analysis revealed not only significant associations of some previously unannotated transcripts with the disease but also intriguing spatial expression patterns across various brain regions, offering a valuable insight into the intricate molecular landscape associated with
neurodegenerative diseases.

Study funded by: The GENCODE Consortium

NOTES

SESSION 2B - NEUROTRANSMITTER RECEPTORS AND SIGNALING

O.22. SYNAPTIC PROTEOME DIVERSITY IS PRIMARILY DRIVEN BY GENE REGULATION OF GLUTAMATE RECEPTORS AND THEIR REGULATORY PROTEINS

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Electrophysiological features of excitatory synapses vary widely throughout the brain, granting neuronal circuits the ability to decode and store diverse patterns of information. Synapses formed by the same neurons have similar electrophysiological characteristics, belonging to the same type. However, these are generally confined to microscopic brain regions, precluding their proteomic analysis. This has greatly limited our ability to investigate the molecular basis of synaptic physiology. Here we introduce a procedure to characterise the proteome of individual synaptic types. We reveal a remarkable proteomic diversity among the synaptic types of the trisynaptic circuit. Differentially expressed proteins participate in well-known synaptic processes, controlling the signalling pathways preferentially used among diverse synapses. Noteworthy, all synaptic types differentially express proteins directly involved in the function of glutamate receptors. Moreover, neuron-specific gene expression programs would participate in their regulation. Indeed, genes coding for these proteins exhibit such distinct expression profiles between neuronal types that they greatly contribute to their classification. Our data is an important resource for exploring the molecular mechanisms behind electrophysiological properties of different hippocampal synaptic types. Our combined analysis of proteomics and transcriptomics data uncovers a previously unrecognised neuron-specific transcriptomic control of synaptic proteome diversity, directed towards the regulation of glutamate receptors and their regulatory proteins.

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NOTES

O.23. MOUSE CORTICAL ASTROCYTES DETECT DOPAMINE VIA NON-COGNATE RECEPTORS

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Astrocytes sense diverse neurotransmitters and neuromodulators and, in turn, orchestrate regulation of neuroactive substances. However, basic physiology of astrocytes in prefrontal cortex (PFC)—a hub for cognitive control that is profoundly influenced by dopamine—is still unclear. Here we characterise divergent signaling signatures in mouse astrocytes of the PFC and primary sensory cortex in vivo, which show differential responsiveness to locomotion. We find that PFC astrocytes express receptors for dopamine but are unresponsive through the Gs/Gi-cAMP pathway. Instead, fast calcium signals in PFC astrocytes are time locked to dopamine release and are mediated by non-cognate α1-adrenergic receptors, both ex vivo and in vivo. Further, we describe dopamine-triggered regulation of extracellular ATP at PFC astrocyte territories, which also depends on α1-adrenergic receptors. Thus, we identify astrocytes as one component of the prefrontal circuitry that responds to dopaminergic inputs, contributing to PFC function via non-canonical neuromodulator/receptor interactions.

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NOTES

O.24. IN VIVO PHOTOCONTROL OF INHIBITORY BRAIN RECEPTORS

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Inhibitory neurotransmission in the brain is mainly mediated by GABAARs. Disequilibrium in their function leads to many sever neurological disorders, such as epilepsy, anxiety, depression. Thus, development of allosteric modulators that would regulate the activity of these receptors with minimized side effects is of great importance. Photopharmacology is a unique tool for these purposes allowing precise spatial and temporal light-driven control of pharmacophores´ activity, and consequently of their target proteins. Pursuing the goal to develop a GABAARs positive photomodulator, we successfully functionalized the benzodiazepine nitrazepam into a light-controllable molecule via extension by a photochromic fulgimide. The molecule that was obtained, Fulgazepam, was demonstrated to be the first photochromic switch-on potentiator of GABAARs and its ability to photomodulate neuronal activity and behaviour was successfully demonstrated in vivo in zebrafish. Next, through azologization of a partial agonist of GABAARs we have obtained a photoswitchable activator of GABAARs. Similarly to the Fulgazepam, it was shown to activate gabaergic currents only after illumination with UV light. Its advantage, comparing to the Fulgazepam, is its higher synthetic accessibility and an activation wavelength shift towards blue part of the spectra – 400-405 nm, which allows better penetration of light into the tissue. We demonstrate that our new photoswitchable GABAARs modulator can photocontrol mechanical sensitivity of mice in vivo. In summary, we have developed a toolbox of photoswitchable modulators of GABARs that can be used for variety of tasks when studying gabaergic neurotransmission. Our latest light-switchable gabaergic molecule is a promising model compound for further therapeutic developments in photopharmacology.

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NOTES

O.25. THREE-PHOTON INFRARED STIMULATION OF ENDOGENOUS NEURORECEPTORS IN VIVO

Sortino R[1,2], Cunquero M[3], Castro-Olvera G[3], Gelabert R[4], Moreno M[4], Riefolo F[1,2,#,] Matera C[1,2,%], Fernàndez-Castillo N[2,5,6,7], Agnetta L[8], Decker M[8], Lluch JM[4,9], Hernando J4, Loza-Alvarez P[3,*], Gorostiza [P1,2,10,*]

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To interrogate neural circuits and crack their codes, in vivo brain activity imaging must be combined with spatiotemporally precise stimulation in three dimensions using genetic or pharmacological specificity. This challenge requires deep penetration and focusing as provided by infrared light and multiphoton excitation, and has promoted two-photon photopharmacology and optogenetics. However, three-photon brain stimulation in vivo remains to be demonstrated. We report the regulation of neuronal activity in zebrafish larvae by three-photon excitation of a photoswitchable muscarinic agonist at 50 pM, a billion-fold lower concentration than used for uncaging, and with mid-infrared light of 1560 nm, the longest reported photoswitch wavelength. Robust, physiologically relevant photoresponses allow modulating brain activity in wild-type animals with spatiotemporal and pharmacological precision. Computational calculations predict that azobenzene-based ligands have high three-photon absorption cross-section and can be used directly with pulsed infrared light. The expansion of three-photon pharmacology will deeply impact basic neurobiology and neuromodulation phototherapies.

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NOTES

O.26. DEEP-PHENOTYPE CHARACTERIZATION OF GRIN1 ZEBRAFISH MODELS, A NEW TOOL TO STUDY GRIN-RELATED DISORDERS

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The NMDA-type family of ionotropic glutamate receptors plays critical roles in neurons and glial cells functions, such as synaptic development, plasticity, neuronal survival. Recently, de novo variants of GRIN genes -encoding GluN subunits of the NMDA receptors- have been associated with neurodevelopmental disorders, defining GRIN-related disorders (GRD), a rare developmental encephalopathy with a clinical spectrum resulting from primary defects on glutamatergic neurotransmission. The genetic heterogeneity underlying GRD aetiology might be addressed using zebrafish larvae model. In this study we have generated and performed a deep phenotypic characterization of deleterious grin1a and/or grin1b zebrafish genes, paralogous to human GRIN1.
Following CRISPR-Cas9 generation of the grin mutant larvae, no alterations on survival rate were detected, grin genes spatio-temporal expression pattern in the main larval stages was characterized and the behavioural phenotypes were investigated in paradigms established using pharmacological acute GRD models. Importantly, zebrafish grin mutants recapitulated GRD-like behavioral alterations, notably affecting the central, autonomic and sensory nervous systems (epilepsy, GI tract and visual impairment, respectively). Ongoing experiments include transcriptomic analysis towards the identification of different alterations in GRD models and, essentially, to unveil new potential therapeutic targets for GRD patients. In summary, our results showed that the comprehensive phenotyping of Zebra-GRIN models will allow to define GRD-like alterations. More importantly, the identified phenotypic readouts will be employed to screen the therapeutic efficacy of repurposed and EMA-approved candidate drugs, to provide personalized therapies for GRD patients.

Ministerio de ciencia e innovacion de España, grant number DIN2018-010024.

NOTES

O.27. COMPREHENSIVE DELINEATION AND PRECISION MEDICINE OF GRIN-RELATED NEURODEVELOPMENTAL DISORDERS, A PRIMARY DISTURBANCE OF THE NMDA RECEPTOR

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Glutamate, the main excitatory amino acid neurotransmitter plays a crucial role in neuronal physiology. Glutamatergic neurotansmission disturbance can result from primary de novo mutations of GRIN genes, encoding for the N-methyl-D-Aspartate receptor (NMDAR) subunits. These rare autosomic dominant conditions cause GRIN-related disorders (GRD, also called Grinpathies), a group of severe developmental encephalopathies. GRD display a clinical spectrum including intellectual disability, hypotonia, ASD traits, motor impairment, epilepsy, and gastro-intestinal distress, in a gene- and residue-dependent manners. Accordingly, as for other channelopathies, the functional annotation of GRIN de novo variants is critical i) to understand GRD pathophysiology, ii) to evaluate potential therapeutic strategies and iii) to define personalised therapeutic approaches. In order to address these issues we have created a multi-angled GRIN cluster initiative, merging computational, experimental, translational, and clinical neuroscience approaches. Bioinformatic analysis was used to build-up a comprehensive and specific GRIN variants database compiling genetic, structural, functional and clinical annotations. This database allowed to define a superimposition structural algorithm drastically increasing GRIN variants annotations with a high predictive likelihood ultimately accelerating GRIN variants functional annotations. Further, an experimental pipeline has been developed for the annotation of GRIN-orphan variants and their functional stratification. Finally, we evaluated and experimentally demonstrated the potential therapeutic benefit of nutraceutical interventions for the rescue of LoF GRIN variants, both in preclinical cellular and animal models, in proof-of-concept GRD cases and currently in the first reported GRD clinical trial. Beyond GRD personalised therapies, our findings open the avenue for for future treatments of genetic and/or environmental conditions perturbing the glutamatergic synapse.

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NOTES

O.28. METABOLIC CHARACTERIZATION OF NEURODEVELOPMENTAL DISORDERS INVOLVING GLUTAMATERGIC NEUROTRANSMISSION

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Despite its crucial role in the identification of new therapeutic targets, the pathophysiological characterization of neurodevelopmental disorders is challenging due to their complex and varied etiologies. Metabolomics offers a promising tool for these pathologies because of its ability to reflect person-to-person differences, from genetics to environmental exposure and lifestyle. In this study, we conducted a semi-targeted metabolomics analysis of cerebrospinal fluid samples from 25 pediatric patients with neurodevelopmental disorders that have a neurotransmission component (Rett syndrome, GRINpathies, and CDKL5- and STXBP1-related disorders), which we compared to five healthy controls. We separated these patients into two groups based on the nature of their alterations in glutamatergic signaling: we classified Rett syndrome and CDLK5-related disorders as hyperglutamatergic, and the GRINpathies and STXB1-related disorders as hypoglutamatergic. We used gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) to detect metabolites related to monoamine (tyrosine and tryptophan) and energy metabolism. Then we performed a statistical analysis consisting on multivariate and univariate approaches, specifically orthogonal projections to latent structures discriminant analysis (OPLS-DA) and Wilcoxon Rank Sum tests with False Discovery Rate (FDR) multiple comparisons correction. We then mapped the selected metabolites to KEGG pathways and found that both groups showed similar decreases in the tryptophan and branched-chain amino acid metabolism pathways. Interestingly, these alterations converge on the LAT1 transporter, which allows crossing of the blood-brain barrier (BBB). To investigate this, we analyzed the brains of mouse models of Rett syndrome and identified decreased expression of the transporter, which provided further evidence of a potential role of LAT1 in the pathology of these neurodevelopmental disorders. Our results shed light on the understudied metabolic component of disorders with glutamatergic

involvement, which could provide valuable information for the design of new therapies and treatments.

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NOTES

SESSION 3A - NEURAL CIRCUITS AND BRAIN PLASTICITY

O.29. EPIGALLOCATECHIN-3-GALLATE PRETREATMENT AS A NEW NEUROPROTECTIVE THERAPY AGAINST POLYMYXIN INDUCED NEUROTOXICITY.

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The appearance of drug-resistant bacteria has made necessary the reintroduction of effective antibiotics with a narrow therapeutic window like polymyxins. It is known that polymyxins are nephrotoxic and causes alterations in the peripheral nervous system. This toxicity is mainly mediated by the generation of reactive oxygen species involving Nrf2 pathway. However, it has been suggested that it may also affect at central level (CNS) while the mechanism of toxicity remains unclear. Hence, this study aims to investigate the effects of polymyxins in the CNS and weather epigallocatechin-3-gallate (EGCG), a potent antioxidant and Nrf2 modulator of green tea, is able to protect against polymyxin induced toxicity. For this study, 7-week-old C57BL/6 female mice were interperitoneally treated with EGCG or saline for one week. Then, polymyxin E or saline was administered at a dose of 9 mg/kg for two weeks, twice a day 8h apart. At the end of the study, 8 behavioral tests related to depression, anxiety, cognition, and coordination were performed. Brains were collected and preserved for immunohistochemistry and Golgi staining. Moreover, to better study the effect on dendritic spines an immunocytochemistry against GFP, PS95 and Synaptophysin in hippocampal primary cultures was performed. Our results demonstrated that polymyxin induced anxiety-, depression-, and cognition-related deficits in mice. This could be explained by a decrease of DCX positive neurons and a reduction of dendritic spines saw in vivo and in vitro. Moreover, polymyxin also caused an alteration of the BBB and an increase of astrogliosis. The pre-administration with EGCG was able to ameliorate all the behavioral related deficits and significantly increase the number of functional dendritic spines.

To sum it up, we have demonstrated that a 14-days treatment with polymyxins causes alterations in the CNS. Furthermore, EGCG pretreatment could be a promising therapy to alleviate polymyxin-induced toxicity and increase its therapeutic window.

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NOTES

O.30. TRESK CHANNEL MODULATES CA3 PYRAMIDAL NEURONS' EXCITABILITY AND HIPPOCAMPAL SYNAPTIC PLASTICITY.

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To comprehend the brain's dynamic physiology, we must understand the mechanisms underlying neuronal excitability, crucial for neurons' electrical activation during synaptic communication. Of particular interest is controlled potassium current leakage via two-pore domain potassium (K2P) channels which finely modulate the intrinsic excitability of neurons. TRESK, the latest discovered channel of the K2P family, has a well-characterized pivotal role in nociception. Intriguingly, it also exhibits widespread expression throughout central nervous system, yet its function within the brain has remained unexplored. We are combining in-situ hybridization RNAscope technique and electrophysiology experiments to examine the contribution of TRESK to hippocampal excitability. We observed TRESK mRNA in excitatory and inhibitory neurons along the CA1-CA3 regions and dentate gyrus. Functionally, field potential and whole-cell patch-clamp recordings in acute slices revealed that TRESK knockout mice exhibit decreased paired-pulse facilitation and impaired long-term synaptic plasticity in the Schaffer Collateral pathway. Additionally, in the absence of TRESK, CA3 pyramidal neurons displayed enhanced excitability via reduced rheobase current and a tendency for a more depolarized resting membrane potential. Accordingly, there was an increased number of cells that were spontaneously active and they had higher firing frequencies compared to control slices. We are currently exploring the role of TRESK in GABAergic cells. Our findings highlight the involvement of TRESK in hippocampal excitability and synaptic plasticity. These data serve as a foundation for future experiments to elucidate the role of TRESK channels in hippocampal intrinsic plasticity, which will provide insights into principles governing the dynamics of neural networks in memory formation.

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NOTES

O.31. LIGHT-DEPENDENT cAMP MODULATION IN ASTROCYTES TRIGGER SYNAPTIC POTENTIATION, HEMODYNAMIC RESPONSES AND BEHAVIOURAL CHANGES IN MICE: ROLE IN HUNTINGTON'S DISEASE.

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Astrocytes are glial cells that participate in crucial processes in brain homeostasis and play a key role in modulating neuronal activity. Numerous astrocytic receptors transduce signals via cAMP although how changes in astrocytic cAMP levels modulate brain activity or animal behaviour is not fully understood. Our goal is to control cAMP in cortical astrocytes using DdPAC, a recently developed phytochrome photoreceptor that increases cAMP upon red light, and evaluate its responses at three levels: a) cellular, by analysing neuronal activity using multi-electrode arrays b) systemic, by measuring in vivo brain hemodynamics with a non-invasive custom-made diffusion wave imaging system c) behavioural, by analysing motor behaviour in a mouse model of Huntington's disease. Activation of DdPAC by red light in cortical astrocytes induced synaptic potentiation in brain slices from WT mice, which was PKA/NMDA-dependent and Ca2+-independent. Then, we observed that DdPAC stimulation induced an increase in blood flow in both WT and HD anesthetized mice. We finally assessed motor behaviour both in WT and HD mice after repetitive DdPAC stimulation and observed distinct effects between genotypes: while motor learning was improved in WT mice, coordination was impaired in HD mice. Altogether, our data demonstrates that light-dependent modulation of cAMP in astrocytes with phytochromes could improve synaptic plasticity in neurons and increase brain activity in specific brain areas. Furthermore, in vivo manipulation of cAMP in astrocytes generated divergent responses in WT and HD mice, which is relevant to further exploring therapeutic opportunities to ameliorate symptoms in neurodegenerative diseases.

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NOTES

O.32. EXPLORING THE DOPAMINERGIC EFFECTS OF S-KETAMINE.

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Ketamine is widely used as anesthetic and analgesic in clinical practice. However, it has been reported toact as an antidepressant at subanesthetic doses, hence recently S-ketamine has been approved by the FDA and EMA to treat treatment resistent depression. Nevertheless, its antidepressant mechanism of action has not yet been fully described. Ketamine is a low affinity non-competitive antagonist of the N- methyl-D-aspartate receptors (NMDAR). However, multiple reports show that the effects of both ketamine enantiomers are also mediated by opioid receptors and other non canonical targets. One key factor to improve ketamine-based pharmacotherapy is to understand and minimize its abuse potential. In rodents, S-ketamine is able to increase dopamine levels in different regions of the brain, including the nucleus accumbens. Moreover, both NMDAR and opioid receptors play synergistic roles within the mesolimbic dopaminergic system, a key system to mediate reward and motivations. The goal of this study was to explore ketamine's modulation of dopamine activity and its effects on mice behavior. First, we explored acute and chronic psychomotor effects of S-ketamine administration in mice and its dependence on opioid receptors. Then, to gain mechanistic insight into the behavioral effects of S- ketamine, we used fiber photometry and optogenetics to study its pharmacological actions on spontaneous or stimulated dopamine and glutamate release. Our results suggest that S-ketamine produces bimodal effects on dopamine dynamics by decreasing its release probability but impairing its re-uptake, an effect shared with other psychostimulants and that can explain its abuse liability.

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NOTES

O.33. DECODING INCIDENTAL ASSOCIATIONS: ROLE OF AMYGDALA IN SENSORY PRECONDITIONING.

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Since our first steps in life we are forming incidental associations with diverse stimuli across various sensory modalities (e.g. olfactory, gustatory) that will influence our future decisions and will facilitate adaptation to environmental changes. Daily behaviour is usually governed by indirect incidental associations among low-salience sensory cues that have never been explicitly paired with a reinforcer. This phenomenon known as mediated learning (ML) can be systematically investigated in laboratory animals through the use of specific behavioral paradigms such as sensory preconditioning protocols.
Here, using "Targeted Recombination in Active Populations" (TRAP2) transgenic mice we have interrogated which are the different brain areas orchestrating the encoding of associations between low-salience stimuli and the expression of ML in an aversive odor-taste sensory preconditioning paradigm. This paradigm can be divided in 3 phases: a preconditioning phase in which two pairings of low salience olfactory-gustatory stimuli are presented; a conditioning phase where one stimulus is devaluated through an injection of lithium chloride and finally, a two choice test where mice are presented with the two different stimuli (ML test). Using TRAP2 mice, we identified neuronal ensembles within the amygdala that are activated during odor-taste associations suggesting an amygdala hub for the encoding of incidental associations. To demonstrate the causal involvement of the amygdala in our sensory preconditioning task, we inhibited this brain region during the preconditioning phase using a chemogenetic approach. Notably, the inhibition of the amygdala during odor-taste associations caused an impairment of ML. Finally, using a combination of anterograde and retrograde tracers in TRAP2 mice, we characterized the circuitry of activated amygdala neuronal ensembles during the encoding of incidental associations. Overall, these findings highlight the amygdala as a pivotal modulator of incidental associations during aversive sensory preconditioning, and shed light for manipulating crucial brain circuits involved in complex cognitive processes.

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NOTES

O.34. EFFECTS OF THE POLY I:C MODEL OF SCHIZOPHRENIA ON THALAMIC INHIBITORY CIRCUITS.

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The mammalian brain is an extremely complex structure, based on the equilibrium between excitatory and inhibitory neurons. In the rodent thalamus, GABAergic interneurons are located almost exclusively in the thalamic reticular nucleus (TRN), where they regulate the excitation of the rest of thalamic nuclei, such as the Ventral Posterolateral (VPL) and Mediodorsal (MD). Many of these interneurons express parvalbumin (PV), a calcium binding protein, and their somata and proximaldendrites are surrounded by a specialized region of extracellular matrix, the perineuronal nets (PNNs). Recent studies have demonstrated that alterations in PNNs can lead to changes in the connectivity of PV+ interneurons. These alterations may contribute to th e etiopathology of certain neuropsychiatric disorder s, such as schizophrenia, an illness in which alterations in inhibitory circuits have been described. Changesin the expression of PV and in the organization of PNNs have been found in patients with schizophrenia and in animal models of this disorder. The aim of this study is to analyze different parameters of PV+ neurons and their surrounding PNNs in the TRN, as well as the density of PV+ synapses onto VPL and MD in male and female rats subjected to the Poly I:C model of schizophrenia, a neurodevelopmental model that consists of a maternal immune stimulation during gestation. Our results demonstrate the existence of a sex related reduction in the density of PV+ cells and PV expression, and differences between the dorsal and ventral regions of the TRN, but not in PV+ neurons' projections to VPL or MD.

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NOTES

O.35. STRUCTURAL PLASTICITY OF DENDRITIC SPINES DURING LONG-TERM SYNAPTIC DEPRESSION.

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Learning and memory are explained at the molecular level by the plastic properties of synapses. Most excitatory synapses are located on dendritic spines, which can undergo both functional and structural changes. Functional and structural plasticity are usually correlated: spines enlarge when they are long-term potentiated (LTP) and shrink when they are long-term depressed (LTD). This is true for LTD induced through NMDA receptors (NMDARs). However, when LTD is induced via metabotropic glutamate receptors (mGluRs), spines do not shrink. To understand how the interaction between NMDARs and mGluR regulates spine shrinkage, we used agonists and antagonists of each type of receptor and modified the activity levels of neurons in rat organotypic hippocampal slice cultures. We observed a novel form of structural plasticity: mGluR-induced NMDAR-dependent (MIND) plasticity. The bidirectional changes in spine morphology associated with this form of plasticity depend on the activity of the neural circuit and the levels of divalent cations. These results suggest that mGluRs could behave as a sensor for synchronic activity that shapes the structure of dendritic spines in the long term.

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NOTES

SESSION 3B - SENSORY AND MOTOR SYSTEMS

O.36. NOT JUST A HIGHWAY: THE ROLE OF THE SPINAL CORD IN RATS' FORELIMB SKILLED FUNCTION

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Traditionally, it has been considered that the correct execution of manual dexterity movements fully depends on the brain, mainly on the motor cortex, and the spinal cord would "simply" be the substrate for the information to be transmitted to the muscle. However, stereotypic features of these movements suggest that some level of control may be exerted from the spinal cord (SC). In this project, we aimed to unveil the presence and location of this hypotetic spinal neuronal network. We inflicted excitotoxic injuries to rats at different cervical SC levels using kainic acid, for exclusively affecting spinal networks while preserving descending commands. Motor deficits were evaluated by comparing the performance, before vs after the intervention, in reaching and grasping accuracy and multiple other behavioural tests of forelimb muscles' function that likely require distinct neuronal networks. Only C3-injured animals showed a significant impairment in the execution of forelimb skilled movements (e.g. reaching and grasping, staircase and horizontal ladder tests). Histological analyses revealed a grey matter and neuronal loss that correlated with the segments of injection. To dismiss the possibility of the partial loss of motoneurons and/or sensory feedback being the cause of the behavioural deficits observed, a subsequent experiment was conducted in which the C3 dorsal and ventral roots were sectioned, therefore eliminating all its direct afferences and efferences but preserving spinal networks' integrity. Those animals did not suffer manual dexterity impairments. Our results suggest the presence, at spinal segment C3, of a neuronal network necessary for controlling reaching and grasping.

Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR)
Fundació La Marató de TV3

NOTES

O.37. 4-DEEP BRAIN RECONSTRUCTION: A 3D-PRINTED MINI-BRAIN BASED ON PLURIPOTENT STEM CELLS DIFFERENTIATION

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Neurodegenerative diseases are characterized by the progressive loss of neurons and other brain cells, leading to cognitive or motor disabilities. Brain disorders have a colossal societal and economic burden since about 1 in 3 Europeans are affected by one of these diseases. Available treatments only slow down disease progression or reduce the symptomatology, unfortunately they fail to entirely stop disease progression. The 4-DBR project proposes to develop a 3D bioprinting strategy to obtain a vascularized minibrain, that can be transplanted into patients affected by neurodegenerative disorders. The first step to develop this new strategy is to develop protocols to differentiate the distinct cellular components from the same human Pluripotent Stem Cell (hPSC) line. Thus, we have recently developed a protocol to obtain striatal neurons as one of the main targets of neurodegeneration in Huntington's disease.
Here we present our recent advances in the production of endothelial cells and pericytes as key components of the blood brain barrier. We show that endothelial cells, expressing both endothelial markers CD31 and von Willebrand factor, can be obtained from hPSC after 10 days of hPSC differentiation. We also show preliminary data of the differentiation of neurons, expressing both neuronal markers MAP2, GABA and CTIP2, on a brain extracellular matrix hydrogel. Finally, we show preliminary data of the co-culture of the different cell components of the blood brain barrier. Our culture protocols for the different cell types serve as a basis in the generation of the bio-printed vascularized mini-brain that can be transplanted in patients.

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NOTES

O.38. DYNAMICS OF NOISE-INDUCED NEURODEGENERATION AND NEUROPLASTICITY IN THE CENTRAL NERVOUS SYSTEM

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Noise-induced hearing loss (NIHL) leads to key changes in auditory structures of the central nervous system (CNS). The present study investigates how cell density, axonal density and/or glutamatergic and GABAergic neurotransmission, could be affected at different time points after acute noise exposure in the central inferior colliculus (CIC) and the ventral medial geniculate body of the thalamus (MGV). Mice were noise-exposed for 3h at high (115 dB) or moderate (90 dB) broadband white noise (5–20 kHz), and unexposed mice were used as controls. Mice were separately investigated 1 day, 7 days, 56 days and 84 days post-exposure. Frequency-specific auditory brainstem responses (ABR) were recorded at 4, 8, 16 and 32 kHz before and after exposure to examine auditory thresholds shifts. Fluorescence immunohistochemistry (FIHC) against NeuN, DAPI, Neurofilament-heavy (SMI312), vesicular GABA transporter (VGAT) and vesicular glutamate transporter 1 (VGLUT1) and 2 (VGLUT2) was performed. Automated Fiji macros were developed to perform quantitative cell counting and fluorescence intensity analysis. Findings showed that ABR thresholds were significantly elevated 7d, 56d and 84d after noise exposure in 115dB-exposed animals when compared to 90dB-exposed or Control mice, suggesting a NIHL phenotype after acute noise delivery. Strikingly, significant elevations in neurofilament density were observed 1d after noise exposure in the CIC and MGV, but not 7d, 56d and 84d post-exposure in 115dB-exposed mice when compared to 90dB or Control animals. Thus, we demonstrated that early excitatory and/or compensatory neuroplastic connectivity changes occur in the auditory CNS as a result of acoustic overstimulation. However, no significant changes in NeuN and DAPI or VGLUT1, VGLUT2 and VGAT expression were observed at any timepoint after noise exposure. Therefore, our data revealed complex adaptive mechanisms present in the auditory CNS structures as a result of noise trauma, which partially explain alterations observed in patients suffering from NIHL.

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NOTES

O.39. TRANSCRIPTOMICS AND PROTEOMICS UNRAVEL MOLECULAR SAFEGUARDS OF KLOTHO IN ALS

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Amyotrophic lateral sclerosis (ALS) is a debilitating disease characterized by progressive muscle weakness and atrophy resulting from the degeneration of motoneurons (MNs). Our research has shown promising results in the SOD1G93A ALS mouse through systemic administration of AAV vectors encoding the neuroprotective protein α-Klotho (KL). To protect MN terminals and neuromuscular junctions (NMJ), we targeted KL secretion in skeletal muscles at early and symptomatic disease stages. Overexpression of KL preserved neuromuscular function, locomotion, and strength in SOD1G93A mice. KL-treated mice showed a higher number of surviving spinal and cortical MNs and reduced glial reactivity in the spinal cord. These positive effects were associated with increased NMJ innervation and muscle mass, and extended survival.

To better understand KL protective mechanisms, we conducted comprehensive RNA sequencing and proteomic studies in the lumbar spinal cord and muscles of KL-treated and mock-treated animals. Transcriptomic and proteomic profiling revealed fewer dysregulated genes and proteins in KL-treated mice compared to controls. KL secretion enhanced markers of synaptic activity and plasticity while promoting axogenesis and axonal transport. Moreover, KL mitigated inflammatory and oxidative responses, reducing mitochondrial dysfunction and inhibiting apoptosis in the spinal cord of SOD1G93A mice. Similarly, KL overexpression in muscles restored gene expression patterns related to muscle contraction, development, fibrosis, and atrophy. In both tissues, novel roles for KL in promoting ubiquitin-mediated proteolysis and correcting RNA disturbances were discovered. Remarkably, KL induced changes in gene expression patterns exclusively in SOD1G93A animals but not in wild-type-treated mice, emphasizing its specificity towards pathological conditions.

In summary, our findings underscore the multifaceted beneficial effects of KL on ALS-associated pathological mechanisms, highlight the advantages of muscle-targeted KL delivery to protect MNs, and provide valuable insights for potential therapeutic interventions in ALS and other neurodegenerative disorders.

Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Generalitat de Catalunya. Ministerio de Ciencia, Innovación y Universidades, Gobierno de España

NOTES

O.40. DECIPHERING THE ROLE OF EXTRACELLULAR MATRIX IN ALS PATHOPHYSIOLOGY

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Amyotrophic lateral sclerosis (ALS) is a devastating adult-onset neurodegenerative disease characterized by the degeneration of upper and lower motor neurons (MNs) that leads to muscle weakness, spasticity, and atrophy. Although extensive number of studies during the last decades have uncovered multiple mechanisms associated to this disease, the frustrating reality is that no effective treatments are yet available. Luckily, the rapid development of OMIC technologies have allowed to identify new promising molecular targets for therapeutic intervention, associated to protein homeostasis, RNA processing or DNA damage, among others. Although these studies have also identified alterations in extracellular matrix (ECM) components in the ALS context, little is known about their contribution to the disease. The ECM is an intricately arranged scaffold of secreted proteins and complex sugars that in the spinal cord regulates the maintenance of the neural functions and plays a central role in key events after injury or in disease. The structural and chemical changes in the composition of the ECM can affect axonal regrowth as well as communication, migration and survival of multiple cellular components that are involved in motor function control in the spinal cord. Nonetheless, studying the ECM is particularly challenging because of its biochemical and biophysical complexity, leading us to hypothesize that its role in ALS might have been considerably underestimated. In this study, we have combined distinct profiling techniques to decipher changes in the ECM components of the spinal cord that specifically occur in ALS. We have also investigated the contribution of ALS-associated ECM proteins to MN degeneration using distinct ECM signal-mimetic strategies on human induced pluripotent stem cells derived MNs. Results obtained so far encourage us to further pursue rationally driven designs of ECM mimetic matrices to better understand ALS pathophysiology and to design novel therapeutic approaches.

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NOTES

O.41. CENTRAL AND PERIPHERAL DELIVERY OF ASO 10-27 INCREASES LIFESPAN, IMPROVES MOTOR FUNCTION AND PRESERVES EXCITATORY SYNAPTIC INTEGRITY AND NEUROMUSCULAR JUNCTION MORPHOLOGY IN A SEVERE MOUSE MODEL OF SMA

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Spinal muscular atrophy (SMA) is a progressive neurodegenerative disease that represents the leading genetic cause of infantile death. It is caused by a severe deficiency of the ubiquitously expressed survival motor neuron (SMN) protein. SMA is characterized by lower motor neuron (MN) loss and muscle atrophy. Nevertheless, there is growing evidence indicating that, beyond MNs, different peripheral tissues and organs are also primarily impacted by SMN deficiency. These include skeletal muscle and lymphoid organs The restoration of SMN protein levels by the antisense oligonucleotide (ASO) nusinersen has transformed the clinical outcome of SMA patients. However, its ability to counteract the disease is still limited.
The aim of this study is to analyze the effect of the nusinersen-like ASO 10-27, administered via different routes, on the SMA alterations found in the central nervous system (CNS), skeletal muscle and lymphoid organs of SMNΔ7 mutant mice, a model of severe SMA. We found that intracerebroventricular and subcutaneous administration of ASO 10-27 impacted on lifespan, improved motor behavior and increased SMN levels in the CNS and peripheral tissues of SMNΔ7 mutant mice. Restoration of SMN levels partially prevented MN deafferentation, a hallmark of SMA pathology. Moreover, ASO treatment also prevented neuromuscular junction denervation and atrophy, and the loss of endplate maturity found in SMA. Similarly, ultrastructural studies showed alterations in the spinal cord and skeletal muscle of SMNΔ7 mutant mice, which appeared partially prevented by ASO treatment. Finally, our preliminary studies in spleen and thymus also suggested a potential involvement of the immune system in SMA pathology. Thus, new approaches combining CNS and peripheral administration of ASO 10-27 with SMN-independent drugs should be investigated to achieve a better outcome in SMA pathology.

Ministerio de Ciencia e Innovación.; La Marató de TV3; Diputació de Lleida.

NOTES

O.42. SUPERIOR COLLICULUS AS A KEY PLAYER IN HUNTINGTON'S DISEASE SENSORIMOTOR COORDINATION DEFICITS: FROM CIRCUITS TO BEHAVIOUR

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Huntington's Disease (HD) is an hereditary neurodegenerative condition characterised by severe motor impairments linked to changes in neural networks in the basal ganglia. Yet, impaired eye movement control i.e. saccadic movements, are also found to be affected from the presymptomatic stages. The superior colliculus (SC) plays a pivotal role in integrating visual perception and saccadic movement coordination. In this regard, recent findings from our group suggest disrupted connectivity between the M2 motor cortex (M2) and SC in the symptomatic R6/1 HD mouse model. To comprehensively understand the involvement of SC in HD pathophysiology, we conducted functional MRI-based connectivity analysis in 17-week-old wild-type (WT) and symptomatic HD mouse models. Our results highlighted significant SC functional connectivity alterations with many cortical regions, including the motor cortex, cingulate, somatosenssory and retrosplenial cortices, also the main SC output periaqueductal grey, among others in HD mice compared to control. Then, we investigated the onset of the sensory-induced symptoms using the robo-beetle test, which relies on SC function, at different stages of disease progression (8-week presymptomatic, 12-week with early motor learning deficits, and 16-week with late motor coordination deficits), alongside ongoing biochemical assessments tracking mutant Huntingtin expression and neuronal expression markers in the SC. Notably, we observed an earlier onset of sensory-induced symptoms in HD females at 8 weeks, while both sexes showed increased tolerance and decreased avoidance and approach responses from 12 weeks onwards. Additionally, employing fibre photometry, we are curently evaluating neuronal activity via fluorescent calcium recordings in the M2 and SC, revealing that visual stimuli activated M2 cortex neurons in WT but not HD mice. Our findings shed light on the gradual temporal progression of SC-related sensorimotor deficits and cellular changes across different HD stages, which is crucial for developing early-phase disease-modifying strategies.

This research is part of SC-HD, NEUROPA and GlioLighT projects. The NEUROPA Project has received funding from the European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No. 863214 (M.M.). The GlioLight project has received funding from the European Innovation Council under grant Agreement 101129705 (M.M.). The SC-HD project has received funding from Horizon Europe Marie Skłodowska-Curie Actions under the action number 101104889 (MK). This study was supported by grants from the Ministerio de Ciencia y Innovación (Spain), under projects no. PID2021-124896OA-I00 (M.M.), no. PID2020-119386RB-I00 (J.A. and M.J.R.) and no. PID2022-1363180B-I00 (G.S.); Instituto de Salud Carlos III, Ministerio de Ciencia, Innovación y Universidades and European Regional Development Fund (ERDF) [CIBERNED, to J.A.], Spain. Also, the project has been supported by María de Maeztu Unit of Excellence (CEX2021-001159), Institute of Neurosciences of the University of Barcelona, Ministry of Science, Innovation, and Universities.

NOTES

SESSION 4A - NEURODEGENERATIVE DISEASES II

O.43. REGIONAL AND CELL-TYPE SPECIFIC MECHANISMS OF CANNABIDIOL BENEFIT IN A MODEL OF MITOCHONDRIAL NEUROPATHY

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Inherited defects in mitochondria-related genes cause mitochondrial disease (MD), with primary central nervous system affectation, severe symptomology, poor prognosis, and no cure available to date. A derivation of the extensively studied Ndufs4-KO mouse model for the most common paediatric MD, Leigh Syndrome (LS), carries the mutation only in GABAergic neurons (GABA-cKO). This GABA-cKO model exhibits a severe epileptic phenotype and autistic behaviours, causally related to neuroinflamma on in the external globus pallidus (GPe).
We have previously shown that cannabidiol (CBD) extends lifespan, fully prevents fatal spontaneous seizures and ameliorates the behavioural deficits in GABA-cKO animals. However, the contribu on of GPe GABAergic neurons to LS pathology and the pharmacological actions of CBD in this brain region are still largely unknown.
In this study, we provide the first molecular evidence supporting the beneficial effects of CBD in the GPe of GABA-cKO mice, using cell type-specific translatomics combined with classical mRNA and protein-level biochemical analyses. Our findings reveal altered expression levels of protein-coding genes related to neuroinflammation, cell state regulation, ion homesotasis, and synaptic plasticity. These gene expression paterns correspond to changes in the levels of key proteins associated with gliosis and innate immunity. Notably, we also observe evidence of post-translational protein lactylation in the GPe of GABA-cKO animals.
Taken together, these results provide novel insights into the molecular mechanisms underlying both the detrimental effects of mitochondria-related mutations and the therapeutic potential of CBD in the context of MD.

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NOTES

O.44. ALTERATION OF HIPPOCAMPAL CB1R CAN DRIVE GABAERGIC DYSFUNCTION LEADING TO COGNITIVE DECLINE IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a rare genetic disease caused by the abnormal repeat expansion of the trinucleotide CAG in the Huntingtin gene. Despite the motor symptoms are the most studied, the onset of cognitive deficits (CD) appears largely before and is the most limiting factor for patients' everyday life. Molecular mechanisms leading to CD are still unknown, and the hippocampus remains an underexplored region. The scarcity of knowledge causes the need for innovative molecular targets and emerge in the absence of treatments for CD. Recently, evidence emphasized CB1R importance in cognition and CD. CB1R is one of the most expressed GPCR receptors in the brain, widely in the hippocampus. Its cell specific expression in GABAergic and glutamatergic cells is essential for cognition. Consequently, the project propose CB1R as a crucial target for CD in HD. Particularly, we aim to: 1) characterize CB1R expression and functions in the hippocampal neurons of HD mouse models R6/1,2) explore CB1R in the hippocampal neuron dysfunction,3) manipulate the receptor in GABAergic/glutamatergic populations,4) assess CB1R agonist WIN 55,212-2(WIN) effect as a possible therapeutic compound for CD in HD. As results, we found that CB1R decreases in the hippocampus of R6/1 mice, at different time points and in both genders, specifically in GABAergic cells. Moreover, GABAergic transmission is altered in R6/1 and viral CB1R increase in GABAergic cells ameliorate CD. Finally, WIN treatment rescues CD, ameliorates CB1R levels, synaptic density, and GABAergic transmission in R6/1 mice hippocampus. In conclusion, the current work confirms CB1R as crucial target for CD in HD, showing that CB1R functional activation in GABAergic cells rescues CD in mice and cannabinoid compounds assume a benefic role on CD.

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NOTES

O.45. CD200-BASED CELL SORTING GENERATES HOMOGENEOUS SUBPOPULATIONS OF TRANSPLANTABLE STRIATAL NEUROBLASTS

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Cell replacement therapy (CRT) holds promise for restoring atrophied tissue in Huntington's Disease (HD) by replenishing degenerating striatal projection neurons (SPNs) that form the direct and indirect striatal outputs. However, the heterogeneity of cell populations resulting from striatal differentiation protocols hinders their clinical translation. Standardizing production procedures is essential to ensure the generation of defined and safe cell products.
Here, we demonstrate that CD200 is a cell surface marker of post-mitotic neuroblasts (NBs). We present a CD200-based immunomagnetic sorting pipeline enabling the selection of striatal NBs derived from human pluripotent stem cells (hPSCs), facilitating a consistent and high-yield enrichment of CD200^high NBs. The resulting cell compositions exhibit hallmark characteristics of SPN-committed NBs, which differentiate into the target SPN-like neurons in vitro. Sorted NBs retain the expression of subpallial markers and survive for at least one month following transplantation into the striatum of immunosuppressed adult mice. Markers of the striatal direct and indirect pathways are detected in the transplanted NBs, emphasizing their potential for a regenerative approach for HD. Moreover, CD200-based selection significantly reduces the presence of proliferative cells in the intrastriatal grafts, minimizing the risk of graft overgrowth and tumor formation in vivo. Since the mature phenotype of CD200^high NBs renders them more sensitive to cell viability challenges related to the transplantation process, brain extracellular matrix (bECM) scaffolds are currently being explored to shield cells during delivery and to enhance engraftment. In preliminary experiments, culturing hPSC-derived neural progenitors on a bECM coating has been shown to increase GABAergic differentiation in a dose-dependent manner.
In conclusion, this work highlights the translational value of CD200-based cell sorting to meet quality and safety requirements for future CRT for HD, by ensuring the presence of specified SPN progenitors while excluding overly proliferative cell types.

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NOTES

O.46. DEVELOPING AN INDUCED PLURIPOTENT STEM CELL (IPSC)-BASED MODEL TO IDENTIFY THE MOLECULAR MECHANISMS GOVERNING THE NEURODEGENERATIVE DISEASE MULTIPLE SYSTEM ATROPHY

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Multiple System Atrophy (MSA) is a fatal neurodegenerative disease, which belongs to the synucleinophaties group like Parkinson's disease (PD) and dementia with Lewy bodies. Its main pathological hallmark is the presence of aberrant accumulation of α-synuclein fibrils in the cytoplasm of oligodendrocytes (glial cytoplasmatic inclusions). The aetiology of MSA is still unknown and its treatment remains symptomatic with very limited efficacy. Therefore, an in vitro model is urgently needed to contribute to the identification of the molecular mechanisms underlying the development and progression of the disease. Most of the current studies have relied on the use of mice models and the development of different methods to recapitulate the disease, which include the transgenic overexpression of the α-synuclein gene under oligodendrocyte-specific promoters and the intracerebral injection of MSA brain extracts. Here, we present a human model based on induced pluripotent stem cells (iPSC). Specifically, we have obtained a collection of fibroblasts derived from MSA patients thanks to the Catalan MSA Registry (CMSAR), representing the two major clinical MSA variants with predominant parkinsonism (MSA-P) and cerebellar dysfunction (MSA-C). Fibroblasts have been reprogrammed to iPSC and subsequently have been differentiated to oligodendrocytes, by recapitulating the key signaling pathways of human development. Significantly, two distinct populations of oligodendroctes have been generated (progenitors and mature), together with neurons and astrocytes derived from the same patients. These neural cultures have been seeded with α-synuclein fibrils (pathological condition), and with α-synuclein monomers (physiological condition), both extracted and amplified from the same MSA patients brains, to trigger the formation of protein deposits reminiscent of those present within the central nervous system of the patients. This approach provides a novel and valuable human cell MSA model to perform transcriptomics, epigenomics and proteomics with the potential to take further our knowledge of the disease pathogenesis

This study was supported by TraP-MEDD (Translational Platform for MSA: Elucidation of Disease-mechanisms and Drug discovery) - FLAG-ERA Joint Transnational Call (JTC) 2021

NOTES

O.47. INCREASED NEUROGENESIS AND BEHAVIOR PERFORMANCE BY IN VIVO REPROGRAMMING

Zaballa S[1], Shen Y[2], Bech X[1], Sancho-Balsells A[1], Ballasch I[1], Alberch J[1], Abad M[3], Serrano M[3], Klein R[2], Giralt A[1] and del Toro D[1]

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Yamanaka factors (YFs) can reverse some aging features in mammalian tissues, but their effects on the brain are largely unexplored. Here, we induced YFs in the brain in a controlled spatio-temporal manner, resulting in increased neurogenesis during development. Embryonic induction of YFs led to cortical expansion, increased number of upper cortical neurons, and improved performance of adult mice on several behavioral tasks. Additionally, controlled YF induction in the adult brain show improvements in a neurodegenerative mouse model. Overall, these results highlight the powerful impact of YFs on neurogenesis and their potential use for brain disorders.

Ministerio de ciencia e inovación de España

NOTES

O.48. STUDY OF CELL TRAFFICKING FOR THE DESCRIPTION OF PERSONALIZED THERAPIES IN PEDIATRIC MOVEMENT DISORDERS

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Cell trafficking alterations are a common feature in many neurodevelopmental diseases. Genetic alterations in these diseases are an exponentially growing clinical entity, from 49 to more than 350 known genes in last 10 years. Autophagy usually stands out as a common altered biological pathway among complex pathophysiology. Our main hypothesis is that autophagy is a common altered biological pathway in cell trafficking pathologies, and the study of its pathophysiology can help us to describe potential alternative therapies.
In our work, we have studied the autophagy flux in cells from two patients diagnosed by NBAS or SYNJ-1 deficiency and their potential modulation through a nutraceutical treatment. We have assessed the autophagic flux using Western Blot and inmunofluorescence to assess the expression of autophagy markers such as LC3B and p62/SQSTM1. This has been done on primary fibroblast cultures and gender/age-matched controls. Even though impaired autophagy is a common trait, we have observed significant patient-specific alterations, and while some patients displayed alterations in the autophagosome biogenesis or their proper recruitment, others showed alterations in the last part of the route, the lysosomal degradative activity. Precise characterization of the autophagy flux has allowed us to define the pathophysiology of these neurodevelopmental diseases.
Moreover, insight on the cell trafficking alterations in each patient has provided the foundation of personalized treatments strategies. We tested spermidine, a nutraceutical component, as an autophagy modulator in vitro for each patient, successfully normalizing impaired autophagy flux in both. This efficacy was evaluated later on the patient in a n-of-1 clinical trial, achieving improvement in some clinical aspects related to motricity and cognition. The study of pathophysiology in our patients with neurodevelopmental movement disorders has been key for the establishments of a personalized therapies trough autophagy modulators. Systematic study of autophagy in cell trafficking disorders will be the base for novel therapeutic options.

NOTES

O.49. AAV9-MEDIATED EXPRESSION OF SECRETED KLOTHO REDUCED SEVERAL AGING-ASSOCIATED PHENOTYPES, IMPROVING COGNITIVE CAPACITIES AND INCREASING LONGEVITY

Roig-Soriano J[1] , Edo A[1] , Verdés S[1] , Sánchez-de-Diego C[2] , Griñán C[3] , Pallàs M[3] , A. Jordan B[4] ,Ventura F[2] , R. Abraham C[5] , Bosch A[1,6,7] , Muñoz P[8,9] , Chillón M1,[6,9,1]

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Advances in health care and quality of life in modern societies have led to an increase in the percentage of the population reaching advanced ages. Aging is one of the main risk factors for pathologies provoking cognitive degeneration, like Alzheimer's disease and dementia, but also physical impairment like sarcopenia and osteoporosis. These conditions are accompanied by suffering, disability, and elevated economic and social costs, thus new therapies are needed to achieve healthy aging. The protein Klotho (KL) has been identified as a promising anti-aging molecule due to its pleiotropic actions, with pro-longevity effects on pathways such as insulin/IGF and Wnt signaling, and inflammatory and oxidative stress modulation. Here, we explored the anti-aging potential of the secreted isoform of this protein in the SAMP8 and C57BL mice, models for accelerated and non-pathological aging, respectively. Systemic and intracerebroventricular delivery of AAV9 efficiently increased concentration of s-KL protein in serum, improving the aging phenotype in different organs analyzed. KL treatment improved fitness in behavioral tests, associated with reduced muscular fibrosis and improved bone microstructural parameters. Cognitive capacities of aged animals were enhanced, which was accompanied by changes in histological markers like Ki67, DCX, Iba1 and GFAP. Furthermore, hippocampal RNAseq transcriptomic analysis suggested s-KL treatment induced an increase in phagocytosis and anti-inflammatory signals in the hippocampus of aged mice. Remarkably, long-term AAV-mediated expression of s-KL lead to a 20% increase in total longevity of C57BL mice. These results show for the first time the pharmacological potential of the increase of s-KL expression to reduce simultaneously the impact of age-associated degeneration in multiple organs.

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NOTES

SESSION 4B - COGNITION AND MENTAL DISORDERS

O.50. β-HYDROXYBUTYRATE COUNTERACTS THE DELETERIOUS EFFECTS OF A SATURATED HIGH-FAT DIET ON SYNAPTIC AMPA RECEPTORS AND COGNITIVE PERFORMANCE

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Objective: The ketogenic diet, characterized by high fat and low carbohydrates, has gained popularity not only as a strategy for managing body weight but also for its efficacy in delaying cognitive decline associated with neurodegenerative diseases and the aging process. Since this dietary approach stimulates the liver's production of ketone bodies, primarily β-hydroxybutyrate, which serves as an alternative energy source for neurons, we investigated whether β-hydroxybutyrate could mitigate impaired AMPA receptor trafficking, synaptic dysfunction, and cognitive decline induced by metabolic challenges such as saturated fatty acids.
Results: In cultured primary cortical neurons, exposure to saturated palmitic acid (200µM) decreased surface levels of glutamate GluA1-containing AMPA receptors, whereas unsaturated fatty acids oleic acid and ω-3 docosahexaenoic acid (200µM) and β-hydroxybutyrate (5mM) increased them. Furthermore, β-hydroxybutyrate countered the adverse effects of palmitic acid on synaptic GluA1 levels and synaptic transmission in cortical neurons, as well as excitability and plasticity in hippocampal slices. Additionally, daily intragastric administration of β-hydroxybutyrate (100 mg/kg/day) for two months reversed cognitive impairment induced by a saturated high-fat diet (49% of calories from fat) in a mouse experimental model.

Conclusions: Our findings underscore the significant impact of nutrients on synaptic function and neuroplasticity, shedding light on the potential of β-hydroxybutyrate to delay cognitive impairments associated with metabolic diseases.

This study was supported by the Spanish Ministry of Science and Innovation (MCIN) (PID2020-114953RB-C22 to NC) co-funded by the European Regional Development Fund, the Biomedical Research Centre in Pathophysiology of Obesity and Nutrition (CIBEROBN) (Grant CB06/03/0001 to NC). The work performed at Universitat de Barcelona was supported by Fondo Europeo de Desarrollo Regional (MINECO-FEDER) (PID2022-139016OA-I00 and PDC2022-133441-I00; to C.G-F. and M.P.), and Generalitat de Catalunya (2021 SGR 00357). P.E.C, E.G, and the work performed at the Albert Einstein College of Medicine was supported by NIH grants R01MH116673, R01MH125772, and R01NS113600.

NOTES

O.51. SEX DIFFERENCES IN FEAR MEMORY PROCESSING IN MICE AND HUMANS.

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It remains unexplored in the field of fear memory whether functional neuronal connectivity between two brain areas is necessary in one sex but not the other. Here, we show that chemogenetic silencing of centromedial (CeM)-Tac2 fibers in the lateral posterior BNST (BNSTpl) decreased fear memory consolidation in male mice, but not females. Optogenetic excitation of CeM-Tac2 fibers in the BNSTpl exhibit enhanced inhibitory postsynaptic currents in males compared to females. In vivo calcium imaging analysis revealed a sex-dimorphic fear memory engram in the BNSTpl. Furthermore, in humans, the single-nucleotide polymorphism (SNP) in the Tac2 receptor (rs2765) (TAC3R) decreased CeM-BNST connectivity in a fear task, impaired fear memory consolidation and increased the expression of the TAC3R mRNA in AA-carrier men but not in women. These sex differences in critical neuronal circuits underlying fear memory formation may be relevant to human neuropsychiatric disorders with fear memory alterations such as posttraumatic stress disorder.

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NOTES

O.52. SMALL RNAs ARE IMPORTANT CONTRIBUTORS IN THE COGNITIVE 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 psychotic or positive symptoms, negative symptoms and cognitive deficits. Currently, antipsychotic treatments can help to manage psychotic symptoms in acute episodes of schizophrenia. However, there is an unmet medical need for the treatment of those cognitive symptoms that appear since the premorbid phase and affect almost 98% of patients. Despite the identification of some molecular pathways that are impaired in schizophrenia, little is known about the possible contribution that small non-coding RNAs (sRNAs) may have in its aetiology and pathophysiology.
To define the contribution of sRNAs to the cognitive symptoms of schizophrenia, we have developed a novel translational model based on the injection of sRNAs from the brain of schizophrenic patients or non-affected individuals into the brain of wild-type mice. Wild-type mice receiving sRNAs from schizophrenic patients showed an impairment in spatial working memory measured by the T-maze test, in comparison with mice receiving control sRNAs. Golgi staining revealed that sRNAs from schizophrenic patients induced a decrease in the spine density of the pyramidal neurons from the CA1 hippocampal region in mice. RNA-seq data showed an altered hippocampal expression of synaptic genes related to dendritic spine development and function. Characterization of sRNA profiles by sequencing of patients' samples will allow us to define potential candidates responsible for these effects.In summary, our results suggest that sRNAs might be sufficient for inducing cognitive symptoms and could play a role as important contributors in schizophrenia.

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NOTES

O.53. CELL TYPE-SPECIFIC EFFECTS OF CHRONIC THC EXPOSURE ON HIPPOCAMPAL PLASTICITY REVEALED BY RNASEQ

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The hippocampus plays a pivotal role in memory formation, and cannabinoids, including delta9-tetrahydrocannabinol (THC), the primary psychoactive compound in marijuana, are known to modulate hippocampus-dependent memories and various forms of synaptic and structural plasticity. In this study, we aimed to elucidate the cell type-specific molecular mechanisms that may underlie some of these effects following repeated THC exposure. We utilized Wfs1-Cre:RiboTag mice expressing hemagglutinin (HA)-tagged ribosomes specifically in CA1 pyramidal neurons. These mice were subjected to daily treatment with THC (10 mg/kg, i.p.) or its vehicle for six consecutive days. We then performed cell type-specific isolation of HA-ribosome-associated mRNAs, followed by RNAseq analysis, which unveiled 215 downregulated and 167 upregulated genes induced by THC exposure.
Subsequent Gene Ontology and KEGG pathway analyses of these differentially expressed genes revealed key biological terms associated with memory, structural plasticity (e.g., dendritic spine organization, dendritic development), and synaptic plasticity (e.g., regulation of neurotransmitter receptor activity, regulation of postsynaptic membrane potential, chemical synaptic transmission, regulation of synaptic plasticity). Intriguingly, among the latter terms, numerous categories related to glutamatergic transmission emerged (e.g., glutamate receptor signaling pathway, glutamatergic synapse, glutamatergic synaptic transmission, ionotropic glutamate receptor activity), which were correlated with decreased expression of glutamate receptor subunits in THC-treated mice. Conversely, our bioinformatic analyses uncovered terms associated with the negative regulation of transcription, consistent with the observed reduced expression levels of immediate early genes in mice chronically exposed to THC. In summary, our results reveal novel molecular pathways dysregulated by repeated cannabis exposure, offering potential targets for improving the medicinal use of cannabis.

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NOTES

O.54. UNDERSTANDING THE NEURO-IMMUNE ALTERATIONS IN SCHIZOPHRENIA: THE IMPLICATION OF THE IKAROS FAMILY.

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Schizophrenia is considered a neuropsychiatric syndrome affecting around 1% of the worldwide population. Most typical symptoms are organized in 3 broad categories; positive (delusions, hallucinations), negative (social withdrawal, anhedonia) and cognitive (impaired working memory, attention deficit), genetic and environmental factors are associated with the expression of these symptoms. However, understanding the etiology of schizophrenia symptoms can be challenging due to their heterogeneity. Lately, alterations in the immune system have been linked to the development of schizophrenia, raising the idea of an aberrant cross-talk between the nervous system (NS) and the immune system (IS) in the context of this syndrome. Nevertheless, the precise underlying molecular mechanism of this miscommunication between these two systems is still insufficiently understood.
We hypothesize that alterations of two members of the Ikaros family of transcription factors (which are crucial molecules for leukocytes maturation and function) in circulating immune cells play a role in the aberrant communication between these two systems (NS and IS) and contribute to the appearance of the most typical symptoms. We have developed genetical and translational models, combining human samples with mouse models, targeting the communication between the IS and the NS. Our models allowed us to identify gene transcription mechanisms in circulating immune cells and specific secreted molecules that may play a role in the development of schizophrenia-like symptomatology. Our models allowed us to identify specific molecules secreted by circulating immune cells that may play a role in the development of schizophrenia-like symptomatology.

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NOTES

O.55. ASTROTACTIN 1 DEFICIENCY RESULTS IN A NOVEL NEURODEVELOPMENTAL DISORDER

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Astrotactin 1 (Astn1) is a glycoprotein expressed during neurodevelopment. It is a neuronal adhesion molecule required for normal migration of young postmitotic neuroblasts along glial fibers. There are no pathologies associated to any variants in ASTN1, but some linkage studies suggest an association between deletions and duplications in ASNT1 and neurodevelopmental disorders such as ASD, ADHD, and OCD.

In this work we report a patient presenting with neurodevelopmental delay, focal epilepsy and autism spectrum disorder characteristics. Genetic analysis revealed the homozygous variant ASTN1 c.1523+1G>T. Since it is the first ever patient diagnosed with a neurological phenotype due to mutations in ASTN1, we performed a whole personalized study of the mutation and its impact in neuronal homeostasis.

We first investigated the effect of the mutation in ASTN1 expression in patient derived fibroblasts, upon confirmation of a detectable expression of the gene in this cell type. We found that the mutation yielded to a severe reduction in the mRNA levels, probably through the activation of the Nonsense-mediated mRNA decay (NMD) pathway. Interestingly, it was associated with an increase in the expression of ASTN2, which interact for glial-guided neuronal migration. Moving forward to the analysis in neuronal cells, we inhibited the expression of ASTN1 by means of shRNA, observing abnormalities in neuronal homeostasis, reinforcing the importance of this gene in neurodevelopment. The associated alterations in neurotransmitter receptors shape the avenue for future personalized treatments.

In summary, this ongoing work is the first report of a patient bearing mutations in ASTN1, confirming its involvement in neurodevelopment and setting the first stone on the description of the natural history of the disease. The personalized study of the pathophysiology gives way to potential therapeutic strategies for this and other neurodevelopmental disorders.

NOTES

O.56. SEX-SPECIFIC CHANGES IN MIRNA PROFILE IN THE PREFRONTAL CORTEX OF DEPRESSED SUICIDAL SUBJECTS.

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AIMS: Major depressive disorder (MDD) is a major public health problem, affecting up to 300 million people worldwide, twice as many women as men. Although depression is known to involve complex interactions between genetic, epigenetic and environmental factors, the molecular mechanisms and brain circuits underlying MDD remain poorly understood. Small RNA molecules, such as microRNAs (miRNAs), have been implicated in neuropsychiatric disorders and numerous studies have identified altered gene expression and anterior cingulate cortex activity in MDD patients. Here, we quantified miRNA expression by small RNA sequencing in the prefrontal cortex (PFC) of individuals with MDD/suicide and healthy controls.
METHODS: miRNA expression was measured in the PFC of 17 depressed suicide patients (7F/10M) and 17 well-matched non-psychiatric controls (7F/10M) using miRNA sequencing. miRWalk (v2.0) database was used to predict miRNA targets and Over-Representation Analysis (ORA) with KEGG pathway was used to identify miRNA-regulated relevant pathways.
RESULTS: We found significant changes in global miRNA expression in the PFC of MDD/suicide subjects. Individual tests for statistical significance ($p \leq 0.05$) revealed that 17 miRNAs were downregulated, while 17 miRNAs were upregulated. In addition, we identified 74 differentially expressed miRNAs in MDD/suicide male and 54 miRNAs in MDD/suicide female compared to their respective healthy controls. Among these, 28 miRNAs were differentially expressed in both subgroups. Thus, miR-4516, a potential suicide biomarker, was upregulated in MDD/suicide male and downregulated in MDD/suicide female. Furthermore, we detected changes in certain miRNAs not previously described in MDD patients, including miR-320d, miR-1299, and miR-151b.
CONCLUSIONS: We identified several miRNAs that showed increased or decreased levels in the PFC of MDD/suicide individuals, some of them showing sex-dependent changes in some miRNAs. Future studies should better characterize functional miRNA-mRNA interactions. Normalizing the levels of these miRNAs using miRNA-loaded nanoparticles may be a therapeutic strategy for depression/suicide, which we are currently working on.

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NOTES

POSTER SESSION - GLIAL CELLS AND INFLAMMATION

P1. DETRIMENTAL EFFECTS OF HIGH-DENSITY STAT3-MEDIATED ASTROCYTIC CORRALLING IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme is the most common and aggressive malignant brain tumor in adults. One of the factors contributing to the progression is the complex tumor microenvironment. Specially, the coexistence of two cell types; tumoral cells and resident astrocytes, sharing genetic and phenotypical backgrounds. This aspect hinders the proper characterization and functional distinction of both cell types during the tumor development. Here, we found a differential expression of the astrocytic markers GFAP and S100β that allowed us to histologically distinguish between tumoral cells and resident astrocytes in GBM human samples and in a GBM mouse model in vivo. Interestingly, the core of the tumor showed a high expression of S100β and a low expression of GFAP, corresponding to the tumoral cells. In contrast, the periphery was rich in GFAP high and S100β low reactive astrocytes that corralled the tumor. Based on that heterogeneity, we evaluated the effects of the JAK/STAT3 pathway inhibition, a master regulator of reactive astrogliosis, using adeno-associated virus encoding SOCS3 in the GBM experimental mouse model. SOCS3 overexpression decreased the percentage of GBM cells expressing S100β in the core of the tumor. Moreover, it induced changes in the peripheral corralling mediated by reactive astrocytes, decreasing the number of GFAP+ cells, but increasing the amount and length of their projections.
Overall, our data demonstrate that STAT3-induced reactive astrocytes, encircling the tumor mass, may play a detrimental role in GBM progression and malignancy. Therefore, targeting reactive astrogliosis could be a potential strategy for GBM therapy.

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P2. NF-KB-MEDIATED TOLERANT PHENOTYPE IN MESENCEPHALIC MICROGLIA: IMPLICATIONS FOR PARKINSON'S DISEASE DOPAMINERGIC NEURODEGENERATION

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Among other factors, microglia are thought to take part in the chronic neuroinflammatory scenario that contributes to the process of neuronal death. However, the factors that maintain the overreactive response of microglia in PD remain mysterious. As we wanted to simulate the chronic neuroinflammatory scenario taking place in PD, we evaluated the response of microglial cells after repetitive pro-inflammatory stimuli induced by LPS and IFN-□ in vitro. In contrast with the over-reactivity of a single input, applying repetitive proinflammatory treatment to microglia reduced the release of nitrites, decreased their cell body size, and most importantly, impeded their effective phagocytic capacity towards dopaminergic cells in co-cultures, indicating the establishment of a tolerant or anergic state. Moreover, the characteristic translocation of NF-κB into the nucleus faded away. Focusing on what processes are occurring inside the nuclei, we also analyzed DNA methylations H3K4me1 and H3K4me3, to understand how NF-κB pathway modifies and modulates the programing of microglial tolerant phenotype. Taking this into account, we explored whether PD patients showed microglial over-reactivity. We observed that phagocytic marker CD16 was significantly increased in PD patients suggesting an exacerbated phagocytic capacity. This phenotype relates with the activation state of microglia in contrast to the establishment of immune tolerance, suggesting that the latter may be hampered in PD. Further exploration for evidence of deficient tolerance in parkinsonian microglia will allow to pinpoint their phenotypic state in this neurodegenerative disease.

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P3. PREVENTING SPATIAL MEMORY DEFICITS IN THE APP/TAU MOUSE MODEL OF ALZHEIMER'S DISEASE THROUGH MICROGLIAL ADP RECEPTOR INACTIVATIO

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Microglia participate in diverse functions crucial to maintaining brain homeostasis, including the modulation of synaptic plasticity and neuronal activity through the microglia-neuron crosstalk. Alterations in this crosstalk have emerged as implicated factors in neurodegenerative diseases such as Alzheimer's disease (AD). Remarkably, microglia have ADP receptors sensitive to ATP released from distressed neurons, triggering microglial chemotaxis and phagocytosis, processes that potentially contribute to neurodegeneration by excessive synapse engulfment and subsequent neuronal dysfunction. To elucidate the role of microglial ADP receptors in AD pathogenesis, our research approach is studying a novel APP/Tau mouse model. Biochemical characterization of the model revealed alterations in microglia, synaptic, neuronal, astrocytic, and inflammatory markers expression. Furthermore, functional assessments demonstrated cognitive amelioration upon ADP receptor blockade, evidenced at early and advanced disease stages, as well as motor coordination dysfunction at advanced disease stages. These findings suggest the exploration of microglia ADP receptors as a promising therapeutic target in combating AD.

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P4. THE ROLE OF MICROGLIA IN TAU PHAGOCYTOSIS AND SPREADING

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Neuronal loss and cognitive impairments in tauopathies are closely linked to the presence of intraneuronal tau aggregates. Increasing evidence suggests that microglia contribute to neurodegeneration, synaptic loss, and tau propagation. We and others have shown that extracellular tau induces neuronal loss in glial-neuronal cultures via microglial phagocytosis and that this is prevented by blocking P2Y6R. Moreover, the inactivation of P2Y6R in P301S Tau mice preserves neuronal integrity and cognitive function, and this is accompanied by reduced tau pathology in the cerebral cortex, indicating that the blockade of microglial phagocytosis reduces neuronal loss and tau pathology in neurodegeneration. Similarly, the pharmacological blockade of a microglial receptor involved in chemotaxis shows cognitive improvements and a reduction of pTau-positive neurons in the hippocampus of a model with tauopathy. To further evaluate the role of these receptors in the phagocytosis of live neurons containing filamentous tau aggregates and identify microglia as the source of extracellular tau being released and seeded in the local environment, we are setting up an in vitro system consisting of co-culturing dorsal root ganglion neurons (DRGn) from adult P301S mice and cortical/hippocampal primary microglia. Our studies support the therapeutic potential of targeting specific microglial receptors involved in the phagocytosis of live neurons in tauopathy and the ongoing mechanistic studies could gather unique information about their potential for translational studies.

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P5. PRE-AMYLOID COGNITIVE INTERVENTION RESTORES MEMORY IN AGED TGF344-AD RATS AND REDUCES MICROGLIAL REACTIVITY, DIFFFERENTLY DEPENDING ON SEX

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Background: Cognitive reserve plays a crucial role in how individuals cope with Alzheimer's disease (AD) cognitive decline. Investigating neurobiological pathways which support cognitive reserve and augment cognitive resilience to pathology is a promising avenue for therapeutic exploration.
Methods: We investigated the effects of early (pre-amyloid deposition) and late (post-amyloid) cognitive stimulation on microglial morphology (proxy for phenotype) and memory in TgF344-AD (TG) and wild-type (WT) rats. Cognitive stimulation was induced by intensive training and testing of Delayed Non-matched to Sample task from 3 to 19 months. Memory was assessed by the novel object recognition (NOR) test at 11 and 19 months of age and microglial morphological parameters by 3D reconstructions of the cells using confocal microscopy. Our study followed a longitudinal design including the effect of sex.
Results: Male 19 months-old TG rats which received pre-amyloid cognitive stimulation showed preserved spatial memory compared to untrained TG rats, as they spent significantly more time exploring the new object vs the familiar ($p<0.05$), as assessed by the NOR test. This effect was not observed in female rats. Regarding microglia, various morphological features assessed through immunofluorescence techniques, demonstrated a transient neuroinflammatory protection in 11-month-old TG rats. No significant effect of treatment was observed in rats which received the late cognitive stimualtion, regarding microglial morphology or recognition memory, indicative that a post-amyloid cognitive intervention may be too late to delay AD pathology.
Conclusion: Our study suggests that early cognitive stimulation promotes a healthier neuroinflammatory cell population, that when combined with other neurobiological process associated with plasticity, leads to sustained neuroplasticity and increased resilience against AD-induced memory deficits. Our data suggests that this type of cognitive stimulation may have greater impact in males compared to females.

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P6. CD300F IMMUNE RECEPTOR-DEPENDENT PHAGOCYTOSIS AND LIPID DEGRADATION IN DEMYELINATING LESIONS OF THE NERVOUS SYSTEM

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The immunoreceptor CD300f modulates several systemic inflammatory processes and participates in the phagocytosis of apoptotic cells. Since its ligands are lipid-like (phospholipids including phosphatidylserine, sphingomyelin, lipoproteins, etc.), it has been postulated that it could act as a sensor of damage-associated molecular patterns (DAMPs). The nervous system has a large amount of lipids, mainly associated with myelin. Microglia contribute to the normal recycling of myelin by phagocytosing debris produced during several processes. We hypothesise that CD300f is important in demyelination processes that generate an overload of lipid debris that must be phagocytosed and degraded. In this regard, we observed that after spinal cord injury, which induces extensive demyelination, CD300f-/- female mice showed worse motor functional recovery, in association with increased lipid accumulation in the centre of injury. With the aim of differentiate whether the effect is due to CD300f function in macrophages recruited to the lesion or in microglia, we generated Cx3cr1+/CreERT2:CD300floxP/loxP mice that allowed CD300f to be deleted only in microglia. We observed that these animals showed worse functional recovery after injury, suggesting that microglial CD300f is relevant during the inflammatory and reparative process. In vitro, CD300f-/- bone marrow-derived macrophage (BMDM) cultures incubated with myelin extracts exhibited reduction in myelin phagocytosis in short periods. However, at longer times, they showed increased intracellular lipid accumulation. Surprisingly, this response observed in vitro is sex-dependent, and is more evident in cells from females than from males.

Banco de Seguros del Estado (BSE), Uruguay; Programa de Desarrollo de las Ciencias Básicas (PEDECIBA), Uruguay; Agencia Nacional de Investigación e Innovación (ANII), Uruguay; Universitat Autónoma de Barcelona (UAB), Spain; Marie Curie Fellow

P7. PROTECTIVE EFFECTS OF ANTI IL-1 TREATMENT IN ISCHEMIC STROKE

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Ischemic stroke is a leading cause of mortality and permanent disability worldwide, with no specific treatment besides revascularization. Severe ischemia can cause life-threatening edema and local and systemic inflammation. As interleukin-1 (IL-1) is a key mediator of inflammation, herein we aimed to evaluate the effects of IL-1 blockade in ischemic stroke using the IL-1 receptor antagonist Anakinra, an IL-1 inhibitor approved for different disorders. Despite IL-1 blockade has been proved beneficial in experimental stroke, it has not been specifically studied in very large brain infarctions. We studied two different models of ischemia in young C57BL/6J mice induced by intraluminal middle cerebral artery occlusion (MCAo) either transiently for 45-min (tMCAo) or permanently (pMCAo), which is considered a model of malignant MCAo infarction. We administered Anakinra subcutaneously as a bolus (24 mg/Kg) followed by an infusion through a delivery pump with either a low or high dose (24 or 120 mg/Kg/day) for 1 or 2 days. We assessed the neurological function of mice using a composite neuroscore and the corner and grip tests, and imaged the brain lesions by MRI. Mice were anesthetized (1 or 2 days after pMCAo or tMCAo, respectively), and blood, lung and brain tissues were obtained for mRNA expression and immunohistochemistry studies. The high dose of Anakinra showed protective effects in both models by reducing infarct volume. In the tMCAo model, Anakinra improved the corner test performance, and the neuroscore showed a positive trend for improvement. Studies of the anti-inflammatory effects of Anakinra in brain tissue using mRNA expression analysis and confocal microscopy are ongoing.

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P8. TYPE I INTERFERON MODULATION OF MICROGLIA FUNCTION IN INFLAMMATORY CONDITIONS

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The immune response occurring after brain ischemia is suggested to play a significant role in the final tissue lesion produced by this kind of insult. As regards microglia, the main brain immune cells, they show both detrimental and protective phenotypes from initial to more delayed stages of ischemia. Shortly after an ischemic insult, microglial cells develop a proinflammatory phenotype which is suggested to play a negative effect on neurons damaged by ischemia. In addition, microglia also present a type I interferon (IFN) response. This response is typical in viral infections, but a neuroprotective effect of the induction of type I IFN response has been proposed in experimental models of brain ischemia. The aim of the present work was to study the effect of type I IFN (IFNβ) on microglial cell function, both in the absence and the presence of a pro-inflammatory stimulus (LPS). Using the murine microglial cell line BV2, we evaluated the inflammatory response, phagocytosis and the presence of lipid droplets, which may have relevant roles in the evolution of the neuronal damage after brain ischemia. In IFNβ-treated microglia, significant inflammatory response or lipid droplet accumulation were not detected, but some increase in phagocytosis was observed. An inflammatory response, phagocytosis and lipid droplet accumulation were clearly induced in LPS-treated microglia. When both stimuli were applied together, the potentiation of pro- and anti-inflammatory markers was detected, associated with a further increase in phagocytosis and lipid droplet accumulation. We also detected IFNβ production in LPS-treated cells. We observed that this endogenous IFNβ contributed to the phenotype of LPS-treated microglia by blocking IFNβ receptor (anti-IFNAR1 blocking antibody). The results obtained show that IFNβ modulates microglial cell function and its response to immune challenges, which may be especially relevant in situations where IFNβ levels increase, such as the immune response associated to brain ischemia.

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P9. ROLE OF PTP1B IN LPS-INDUCED NEUROINFLAMMATION MICE MODEL

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Neuroinflammation is a key contributor to the pathogenesis of neurodegenerative diseases, exerting an impact on neural circuits crucial for learning and memory within the hippocampus. Among phosphatases, protein tyrosine phosphatase-1B (PTP1B) stands out since it acts as a critical modulator of numerous signaling cascades involved in inflammation and synaptic processes. In this context, PTP1B has been proposed to modulate neurological disorders where inflammation plays a significant role. Thus, the aim of this study is to elucidate the potential protective role of PTP1B ablation in a lipopolysaccharide (LPS)-induced inflammation mouse model.

For that, 2-month-old wild-type (WT) and PTP1B-deficient (PTP1B -/-) C57BL/6J male mice have been subjected to intraperitoneal administration of LPS or saline solution at a single dose of 1 mg/kg. Subsequently, behavioral tests and molecular assays were conducted to assess memory capacity and inflammation amelioration.

The results of this study revealed that the absence of PTP1B led to the improvement in depressive-like behavior and long-term recognition memory, as evidenced by the Forced Swimming Test and Novel Object Recognition Test, respectively. In accordance, the lack of PTP1B exhibited a reduction in dendritic spines alterations, among other synaptic hallmarks associated with memory impairment. Furthermore, analysis of GFAP and IBA1 expression indicated decreased astrocyte and microglia reactivity, respectively, suggesting attenuated gliosis in PTP1B-deficient animals compared to the WT LPS-treated group.

In conclusion, the deficiency of PTP1B confers neuroprotection against inflammation, thereby improving cognitive functions. Consequently, this study has showed the relevance of PTP1B contribution in neuroinflammation and neurodegeneration, highlighting the potential advantages of PTP1B inhibition. These findings could open new opportunities for the development of therapeutic strategies to improve cognitive function in inflammatory conditions.

Universitat de Barcelona ; Ministerio de Ciencia e Innovación ; CIBERNED

P10. MONOCYTE-DERIVED MICROGLIA-LIKE CELLS AND IMMORTALIZED CELL LINES AS IN VITRO MODELS OF HUMAN MICROGLIA

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Microglial cells play various roles in the healthy CNS, and are particularly relevant in pathological conditions. Most of our knowledge about microglial biology has been obtained using in vitro strategies, the most popular being primary cultures from rodent neonates. However, there are examples of different responses between human and rodent microglia. A striking case is the NO· production by NOS2. Rodent microglia treated with TLR agonists and/or proinflammatory cytokines upregulates NOS2 expression and NO· production which plays an important role in neuroinflammation-induced neurodegeneration. In contrast, human microglial cells under the same stimuli produce undetectable or very low levels of NO·. These differences highlight the importance to develop robust models to study human microglia. To date, there is not a gold standard protocol to study human microglial cells in vitro. The model that best reproduces the in vivo microglial phenotype is the primary culture of adult human microglia but, unfortunately, it yields limited cell numbers, and presents ethical and practical challenges. Alternative methods include human microglial cell lines, and the differentiation of readily available adult human cells, such as iPSCs or monocytes, into cells with a microglial phenotype, often called microglia-like cells. We present here results from our experience with microglia-like cells derived from adult human monocytes and preliminary data the immortalized human microglial cell lines SV40, C20 and HMC3. We have tested various culture conditions and studied morphological changes, phagocytosis, and lipid droplet formation and the expression of proinflammatory genes and interferon-stimulated genes in response to LPS and IFNbeta. Our data show that these models reproduce most, but not all, murine microglial responses and highlight the relevance of human primary microglial cultures as the gold standard to investigate the inflammatory component of brain diseases.

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P11. GLUTAMATERGIC NEURONAL TRANSMISSION REGULATES ASTROCYTIC FATTY ACID METABOLISM

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Energy metabolism in the brain accounts for approximately 20% of the body's oxygen consumption being glutamatergic transmission 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. Lactate released by astrocytes can be oxidatively metabolized by neurons. However, little is known about the interplay between neuronal activity and astrocytic metabolism concerning other energetic sources. Here, we made use of primary cultured rat astrocytes to show that glutamatergic neurotransmission also decreases mitochondrial fatty acid beta-oxidation and oxygen consumption thereby increasing the local oxygen availability. Such effect is independent of brain area and reproduced in human cortical astrocytes. Mechanistically, glutamate inhibition of fatty acid metabolism involves GLAST/EAAT1 transporters but not activation of glutamate metabotropic nor NMDA or AMPA receptors. Glutamate, which alters mitochondrial pH, does not affect Acetyl-CoA Carboxylase phosphorylation status, responsible to produce malonyl-CoA, the endogenous inhibitor of fatty acid oxidation. Importantly, this glutamatergic-induced metabolic adaptation ensures astrocytic survival. In conclusion, glutamatergic regulation of astrocytic use of glucose and fatty acids provides the brain with metabolic flexibility able to perfectly couple and fulfill neuronal activity and astrocytic functions.

Agencia Estatal de Investigación, Ministerio de CIencia, Innovación y Universidades, Gobierno de España.

P12. DEVELOPMENT OF A BLOOD-BRAIN BARRIER MODEL IN VITRO TO ASSAY THE EFFECT OF ANTI-INFLAMMATORY TREATMENTS IN ISCHEMIC/INFLAMMATORY CONDITIONS

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The blood-brain barrier (BBB) is the first line of defense to impede the entrance of pathogens and it controls the transport of selective molecules to the brain. Cerebral ischemia induces cell death and inflammation that subsequently damage endothelial cells, a fundamental component of the BBB. Inflammation in endothelial cells triggers the breakdown of the BBB and exacerbates the ischemic injury. Therefore, it is fundamental to preserve the integrity of the BBB to improve the recovery post-stroke. In the last decades, endothelial cells gained interest as a new target for stroke. In order to unravel mediators of the human BBB vulnerability and to assay the effect of anti-inflammatory treatments on the barrier functionality, we have been developing a model of BBB in transwells with cell lines of human astrocytes and endothelial cells from the brain microvasculature (hBMVEC) and characterizing their response to ischemia/inflammation. We first compared the effect of oxygen and glucose deprivation (OGD), inflammation (with lipopolysaccharide: LPS) and OGD+LPS to select the challenge that better mimics barrier disfunction. Under our experimental conditions, inflammation or OGD alone mildly affected BBB permeability, whereas OGD+inflammation exerted a synergistic effect compromising BBB integrity. We are currently analyzing the expression of pro-inflammatory cytokines, adhesion molecules and proteins released by endothelial cells exposed to these challenges.

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P13. CANNABINOID CB2 AND SEROTONIN 5HT1A RECEPTOR COMPLEX ROLE IN AN HYPOXIA ISCHEMIC ANIMAL MODEL

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Cannabidiol (CBD) is a phytocannabinoid with potential in one of the most prevalent syndromes occurring at birth, the hypoxia of the neonate. CBD targets a variety of proteins, like cannabinoid CB2 and serotonin 5-HT1A receptors. These two receptors may interact to form heteromers (CB2-5HT1A-Hets) that are also a target of CBD. The aim of this paper was to look for the effect of CBD and of another phytocannabinoid used for comparison, cannabigerol (CBG) on the structure, function and expression of receptor heteromers. First of all, we noticed that both CBD and CBG affect the structure of the heteromer, but in a qualitatively different way, namely CBD but not CBG increased the apparent affinity of the receptor-receptor interaction. The two cannabinoids were able to affect the signaling triggered upon receptor activation by cannabinoid and serotonin agonists. We next assessed the expression in a cell model subjected to glucose oxygen deprivation (GOD). CBD and, to a lesser extent, CBG, were able to revert the upregulation of heteromers occurring when GOD was induced. Importantly, the expression of the CB2-5HT1A-Het is significantly increased in brain slices from lesioned animals and GOD primary striatal neurons. Importantly, administration of CBD significantly reduced the expression of such complexes, which are upregulated in the animal model of hypoxia-ischemia. The results suggest that the benefits of CBD in the hypoxia of the neonate are mediated by acting on CB2-5HT1A-Hets and by reducing the aberrant expression of the receptor-receptor complex in hypoxic-ischemic conditions.

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P14. A2AR-CB2R HETEROMER IN STROKE: A POTENTIAL THERAPEUTIC TARGET THAT FAVOURS THE NEUROPROTECTIVE PHENOTYPE OF MICROGLIA.

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Stroke is a leading cause of long-term disability worldwide with substantial economic costs derived from patient care and treatment. Hence, there is an urge for finding novel therapies to treat disability associated to stroke. This is the main reason intensive research has focused on the discovery of key molecules involved in the functional prognosis of patients after stroke. Adenosine A2A and CB2 are receptors that have proven to be a new therapeutic target to consider in treatment of stroke. A common feature of these receptors in neurons is that their expression is upregulated in neuroinflammation process. On the one hand CB2R show a neuroprotective role, delaying neurodegenerative processes after an infart, while A2AR is associated with neuroinflammation, since, blockade of A2AR show neuroprotection in different neurodegenerative processes. One possible mechanism underlying this neuroprotection could be the control of neuroinflammation, that is associated with brain damage. Another possibility could be associated to cannabinoid system regulation. We here show a close interrelationship between those receptors in transfected cells and neuronal primary cultures where they are able to physically interact and affect the signaling of each other due to allosteric interaction within an A2A-CB2 receptor heteromer (A2A-CB2Het). Particularly relevant is the A2A-CB2Het expression increase in samples from an ischemic with hypoglycemia mice model. The most relevant finding, confirmed in both heterologous cells and in primary cultures of neurons, was that blockade to A2A receptors resulted in increased CB2R-mediated signaling. This heteromer-specific feature suggests that A2AR antagonists would potentiate, via microglia, the neuroprotective action of endocannabinoids with important implications for an AD therapy.

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P15. THE INFLUENCE OF CA2+-PERMEABLE AMPA RECEPTORS ON CALCIUM DYNAMICS IN HIPPOCAMPAL ASTROCYTES.

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Calcium signaling is key to understanding the active involvement of astrocytes in the nervous system. Over the past few decades, emphasis has been placed on the role that ion channels play in this phenomenon. In this regard, AMPA receptors (AMPARs) play an important role in glutamatergic neurotransmission, being essential in learning and memory processes. However, they are also present in glia and, while the presence of GRIA genes in hippocampal astrocytes is well established, the functional expression of AMPARs in these astrocytes remains a subject of debate. Primary astrocytic cultures were prepared from hippocampal tissue and their purity was confirmed using specific astrocytic markers. Calcium-imaging experiments were performed by loading astrocytes with fura-2, a calcium-sensitive dye, to measure intracellular calcium changes upon astrocyte stimulation. Western blotting experiments were performed to confirm the protein expression of AMPAR subunits, and patch-clamp recordings were conducted in whole-cell configuration to investigate AMPAR functionality. Our results demonstrated significant changes in intracellular calcium levels upon AMPA stimulation, providing evidence for the functional presence of AMPARs in hippocampal astrocytes. Western blot analysis confirmed the expression of GluA1, GluA2, and GluA4 subunits of AMPARs in hippocampal astrocytes while patch-clamp recordings revealed distinct subpopulations with different kinetics and steady-state current, further supporting the presence of functional AMPARs. Additionally, we evidenced that calcium permeable AMPARs are present eliciting fast calcium signaling and we observed that AMPA stimulation is capable to lead a cross-talking between astrocytes involving ATP release that augment the calcium response. In conclusion, our findings contribute to the ongoing discussion regarding the functional expression of AMPARs, establishing their presence and role in calcium signaling in vitro. Further investigation is warranted to fully understand the contributions of hippocampal astrocytic AMPARs to glutamatergic neurotransmission.

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POSTER SESSION - NEURODEVELOPMENT AND RELATED DISEASES

P.16. STUDY OF THE RHOGTPASE RND3/RHOE IN THE FORMATION OF CORTICAL FOLDS DURING THE DEVELOPMENT OF THE BRAIN USING GENETICALLY MODIFIED MOUSE MODELS

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Little is known of the intracellular machinery that controls the motility of newborn neurons. Rnd proteins are unique due to their inability to switch from a GTP-bound to GDP-bound conformation. Even though our previous data have revealed that Rnd3/RhoE is an important regulator of axon guidance for TCAs projection and of cortical folding during brain development in vivo where Rnd3/RhoE can plays a key role in the control of the folding of the mammalian cerebral cortex, a crucial step related to cortical expansion during evolution.
Based on these data we put forward these primary results with the aim to address the exact role of Rnd3/RhoE in these two aspects of brain development; study the molecular mechanisms regulated by RhoE/Rnd3 involved in the correct projection of thalamocortical axons and characterize the role of Rnd3/RhoE in cerebral cortex folding and investigate the molecular mechanisms involved in this process.
The compiled data from this study implies that Rnd3 may not be a traditional small GTPase. The basic role of Rnd3 is to report as an endogenous antagonist of RhoA signaling-mediated actin cytoskeleton dynamics, which specifically contributes to cell migration and neuron polarity. Therefore, a better understanding of the function of Rnd3 under different physiological and pathological conditions, through the use of suitable models, would provide a novel insight into providing important molecular clues of how our nervous system is built and functions.

Instituto de Investigación Biomédica de Lleida Fundació Dr. Pifarré; Universitat de Lleida; Generalitat de Catalunia; Ministerio de Economía y Competitividad

P.17. UNFOLDING THE CEREBRAL CORTEX. THE STUDY OF RND3 KNOCK-OUT BRAINS MAY LEAD TO THE DISCOVERY OF NOVEL MOLECULAR MECHANISMS OF CORTICAL FOLDING

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Rnd3 gene is a member of the Rho Family of GTPase3 encoding for a protein that competes with RhoA binding to downstream targets causing inhibition of stress fiber formation. This effect on cellular cytoskeleton might be involved in brain folding development of the cortex in mammals. Our project focuses in generating mutations in the Rnd3 gene phosphorylation sites to see what the impact on the murine brain is. More specifically, the poster that I will present will be about a full Rnd3 knockout (Tm1D) and its morphological and developmental changes in the murine brain.

Generalitat de Catalunya

P.18. RHOE/RND3: MOLECULAR MECHANISMS INVOLVED IN THE DEVELOPMENT OF CORTICAL FOLDS DURING MOUSE BRAIN DEVELOPMENT

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Cortical folding is a prominent trait observed in larger brains, serving to compact an expanded cortex into the confines of a limited cranial volume while facilitating proximity among cortical regions with related functions. Although often associated with humans and primates, cortical folding is not exclusive to these groups; it is a recurring phenomenon across various mammalian species. This leads to the classification of mammalian brains into two categories: gyrencephalic species, possessing folded brains like most primates (including humans), and lissencephalic species, characterized by smooth-surfaced cortices, such as mice. Despite the intricacies surrounding the formation of cortical folds, it is evident that this process is complex, genetically regulated, and involves various developmental stages, including proliferation, generation of specific progenitor cells, tangential expansion of progenitor cells, regional cortical growth discrepancies, migration, and differentiation. Notably, in gyrencephalic species, the subventricular zone (SVZ) is subdivided into inner (ISVZ) and outer (OSVZ) regions, augmenting the progenitor cell pool, which is essential for cortical size increase and evolutionary expansion. The abundance of OSVZ progenitors, particularly basal radial glial cells (bRGCs), appears crucial for cortical fold development. Unlike apical radial glial cells (aRGCs) in the ventricular zone (VZ), which possess both apical and basal radial glial fibers attached to the ventricular and pial surfaces, respectively, bRGCs in the SVZ exhibit only basal processes. Our current research endeavors to investigate the role of a specific gene, 'Rnd3,' believed to be implicated in brain folding formation, through both in vivo and in vitro approaches.

Ministerio de ciencia e innovacion; Generalitat de catalunya

P.19. INVESTIGATING THE NEUROPROTECTIVE POTENTIAL OF GESTATIONAL MELATONIN TREATMENT IN AN IN VIVO RABBIT MODEL OF INTRAUTERINE GROWTH RESTRICTION

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Intrauterine growth restriction (IUGR) involves a significant reduction in fetal growth rate and is known to adversely affect brain development. This study aims to assess the neuroprotective potential of gestational melatonin (MEL) treatment in an in vivo IUGR rabbit model. IUGR was induced by surgically ligating the uteroplacental vessels of one horn in pregnant rabbits, with the other horn serving as the control (CNT). Dams received either placebo (PLA) or MEL orally from gestational day (GD) 25 to 30. Cesarean section was performed at GD 30 for pup delivery. Placental histopathology was examined, followed by quantification of oligodendrocytes, evaluation of neuronal arborization, and DAB staining of melatonin receptors in the brain. A significant decrease in body weight and percentage of survival were observed in IUGR groups in comparison to CNTs, confirming the successful induction of the model. Placenta analysis revealed a higher percentage of ischemia phase 2 in the decidua in IUGR compared to CNT, and a decreased percentage of calcifications in MEL-treated groups compared to PLA. Regarding brain analysis, the number of oligodendrocytes tended to decrease in the PLA-treated IUGR group compared to CNT, but this effect was reversed with MEL treatment. A decrease in soma area and primary dendrites length was observed in the IUGR group compared to CNT. Regarding MEL receptors, PLA-treated IUGR showed a significant reduction compared to CNT, with trends towards increase after MEL administration. Notably, a dual response to treatment was observed in this regard, with a decrease in the CNT group and an increase in the IUGR group. To sum up, MEL demonstrates a beneficial effect on the IUGR brain, particularly in increasing the number of oligodendrocytes. These findings highlight the need for further investigation to elucidate the underlying mechanisms.

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P.20. HUMAN STEM CELL-DERIVED NEURONAL CULTURES TO MODEL NEURODEVELOPMENTAL DISORDERS

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Animal models have been widely used for the study of neurological disorders. However, these models may not fully mimic the physiopathology of human brain diseases. Hence, human stem cell-derived models have emerged as a promising alternative. This approach has been strengthened by advancements in gene-editing and cell reprogramming techniques, as well as neuronal activity monitoring technologies.
In the present project we aimed to develop human induced pluripotent stem cell (hiPSC)-derived neuronal models and evaluate the presence of functional network connectivity alterations. We used cellular genetic models and corresponding isogenic controls, genetically engineered with CRISPR/Cas9, for neurological disorders that manifest during neurodevelopment (e.g., lysosomal storage diseases). Human fibroblasts obtained from patients and/or healthy donors were reprogrammed into induced pluripotent stem cells (iPSCs) and afterwards differentiated into cortical neural cells using different strategies. Firstly, a transcription factor (TF)-based differentiation protocol was applied by overexpressing lineage-specific TFs, allowing fast and efficient conversion of iPSCs into highly pure populations of induced neurons and astrocytes. Secondly, iPSCs were neuralized into cortical progenitors called long-term neuroepithelial-like stem (lt-NES) cells. Those were then used to give rise to neural cultures with cortical identity that reached high levels of functional maturity and resembled the development of human brain cortex. Furthermore, we also generated cortical organoids from lt-NES cells. All these models were modified, using viral vectors, to express genetically encoded calcium indicators (GECIs) for dynamic monitorization of neuronal activity in developing networks with cellular resolution. Advanced network tools based on intracellular calcium imaging were also applied in order to compute functional connectivity traits.
Our findings demonstrate that hiPSC-derived cultures are excellent models to have a better understanding of neuronal disorders, and show applicability for future studies on disease mechanisms and drug development.

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P.21. BEHAVIORAL AND DEVELOPMENTAL CONSEQUENCES OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN ZEBRAFISH: A COMPARATIVE ANALYSIS

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Recently, nonsteroidal anti-inflammatory drugs (NSAID) have gained attention due to their widespread presence in the environment. Although their pharmacological mechanism of action is well-known, the potential negative impacts on neurological development remain poorly understood. In this study, we use the zebrafish embryo and larvae model as alternative for traditional developmental neurotoxicity testing (DNT). In order to understand the effect of NSAIDs on zebrafish behavior, we used six different NSAIDs which were either COX-1 selective, COX-1 biased, non-selective or COX-2 selective. Zebrafish embryos were exposed to increasing concentrations of these NSAID (SC-560, NS-398, indomethacin, oxaprozin, celecoxib and diclofenac). Developmental impact of NSAIDs was initially assessed using the FishInspector software, followed by the determination of suitable concentrations for behavior analysis. Spontaneous tail coiling (STC) was examined after 2 day post-fertilization (dpf) and thigmotactic response at 3 dpf. Furthermore, the results obtained were compared with their acute thigmotactic effect at 3 dpf to assess direct effect on nervous system. Indomethacin showed the highest teratogenic index of 7.3. Almost all non-selective NSAID showed decreased head size with sensitivity ratio above 2.4. The most potent chemicals, which mainly caused embryotoxic effects, were selective NSAIDs. Only zebrafish embryos exposed to COX-1 selective or COX-1 biased inhibitors showed an increased frequency of STCs. Oxaprozin exposure significantly increased thigmotaxis after an acute exposure, while indomethacin and SC-560 showed increased thigmotaxis during developmental exposure after 3 days. STC analysis revealed that inhibition of COX-1 induced a hyperactive phenotype in zebrafish. From the thigmotaxis assays, results suggest that inhibition of COX-1 appears to induce acute and developmental anxiety-related alterations in zebrafish larvae. Inhibiting a specific isoenzyme may lead to increased neurotoxicity, prompting further exploration and highlighting the importance of studying NSAIDs' effects on the nervous system.

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P.22. DIFFERENTIAL ROLE OF JNK ISOFORMS IN CORTICOGENESIS

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The mammalian cerebral cortex contains different types of neurons distributed across six layers that are regionally organized in specialized areas. Cortical layering are formed during embryonic development in an inside to outside manner. At the beginning of corticogenesis, the cortical progenitors, Radial glial cells (RGC), derived from embryonic neuroepithelium, proliferate and gradually differentiate to generate neurogenic progenitors. In addition, they play a role in neuronal guidance, facilitating the neuronal position in the different layers.
Different studies supported that c-Jun N-terminal kinases (JNK) belong to the Mitogen-Activated-Protein- Kinase (MAPK) that are involved in regulating the migration of cortical interneurons, as well the timing of their arrival in the different cortical layers. Specifically, dysregulation of these JNK pathways results in aberrant migration that will influence the formation of cortical circuits. Due to Jnk's function and its potential involvement in corticogensis we aimed to investigate whether the final distribution of six cortical layers in adult mice is influenced by JNK isoforms (JNK1, JNK2 and JNK3).
The cortical layers distribution in motor and somatosensorial areas, were analyzed in adult knockout (KO) mice for the different JNK isoforms (Jnk1-/-, Jnk2-/-, Jnk3-/-) in comparison to wild-type mice. A double immunofluorescence was performed using specific markers for the upper (CUX-1) and lower layers (Ctip2). The results showed differences in the organization of cortical layers between JNK KO, evidencing that JNK controls the cortical layer distribution in a specific isoform manner. In particular, Jnk3-/- mice showed greatest differences in cortical layer distribution in contrast to what happen in Jnk1-/- and Jnk2-/- mice, that seems to perform an opposite role. The data obtained conclude that JNK isoforms may participate differently in corticogenesis.

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P.23. PROFILING TIGHT JUNCTIONS PROTEIN EXPRESSION IN BRAIN VASCULAR MALFORMATIONS

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Background: Recent studies suggest that chronic inflammation and blood-brain barrier disruption (BBBd) play a key role in the clinical course and the risk of bleeding of brain arteriovenous malformations (bAVMs)[1,2]. The tight junctions (TJs) are complex endothelial transmembrane proteins with a significant contribution to BBBd. Their expression can be modified in response to pathophysiological and hemodynamic stressors[3,4]. In this study, we hypothesized that bAVMs display a different TJs' pattern than other vascular malformations and than normal brain tissue.
Methods: We studied the expression of the TJs Claudin-5 and Occludin. Human specimens of surgically resected cerebral cavernoma malformation (CCMs) (n=9), bAVMs (n=17) and the parenchyma of its perilesional zone were analyzed by Western blotting, TEM and confocal microscopy after immunofluorescence staining.
Results: The tight junctions were decreased in bAVMs specimens compared to its perilesional zone. Immunofluorescence analyses revealed a statistically decreased expression of both claudin-5, and occludin in the blood vessels of bAVMs compared to the corresponding perilesional zone. This difference was not seen in CCMs. The expression of occludin and claudin-5 was higher in the perilesional zone of bAVMs than in the perilesional zone of CCMs. Similar results were observed in WB analysis.
TEM images provide evidence of disrupted connectivity between endothelial cells of bAVMs, indicating the loss of tight junctions, which aligns with the findings from IF and WB assays.
Regarding the comparison between samples obtained from ruptured and unruptured bAVMs, our study found significant differences in the expression of TJ proteins, in the perilesional zone of bAVMs, suggesting that the bleeding are damaging the parenchyma around the malformation.
In conclusion, alterations in tight junction proteins in bAVMs can disrupt the BBB, leading to increased permeability and allowing harmful substances to enter the brain. This can contribute to neurological symptoms and damage associated with AVMs

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POSTER SESSION - NEURODEGENERATIVE DISEASES I-II

P.24. UNRAVELING THE IMPACT OF HIPPOCAMPAL ASTROCYTES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Astrocytes are implicated in several cellular and synaptic mechanisms that play a crucial role in different pathophysiological and behavioral phenotypes linked to Alzheimer's disease (AD). Our aim is to elucidate the contribution of astrocytes in different AD phenotypes, opening a window to new treatments against cognitive deficits in AD.
In this study, we used male and female APP/PS1 mice as a model of AD. First, we set up a behavioral analysis combining EzTrack® and DeepLabCut™ with handmade Python scripts. Notably, we observed sex- and genotype-dependent effects in different cognitive domains. Second, we used in-vivo fiber photometry to investigate whether astrocyte calcium dynamics are linked with memory alterations. Animals at five-months-old were operated and infused with pZac2.1 gfaABC1D-cyto-GCaMP6f virus in dorsal and ventral hippocampus. After 4 weeks, animals performed the behavioural paradigms in a within-subjects design (novel object recognition and light-tone sensory preconditioning). Our preliminary data suggest sex- and/or genotype-dependent differences in hippocampal calcium dynamics at different phases of the behavioral tasks.
Overall, this data suggests a direct link between astroglial calcium dynamics and the behavioral differences found in an AD' animal model, which should be further confirmed with causal experiments.

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P.25. THE EXPRESSION AND FUNCTIONALITY OF CB1R-NMDAR COMPLEXES ARE DECREASED IN A PARKINSON'S DISEASE MODEL

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One of the hallmarks of Parkinson's disease (PD) is the alteration in the expression and function of NMDA receptor (NMDAR) and cannabinoid receptor 1 (CB1R). The presence of CB1R-NMDAR complexes has been described in neuronal primary cultures. The activation of CB1R in CB1R-NMDAR complexes was suggested to counteract the detrimental NMDAR overactivation in an AD mice model. Thus, we aimed to explore the role of this receptor complex in PD. By using Bioluminiscence Resonance Energy Transfer (BRET) assay, it was demonstrated that alpha-synuclein induces a reorganization of CB1R-NMDAR complex in transfected HEK-293T cells. Moreover, alpha-synuclein treatment induced a decrease in the cAMP and MAPK signaling of both CB1R and NMDAR not only in transfected cells but also in neuronal primary cultures. Finally, the interaction between CB1R and NMDAR was studied by Proximity Ligation Assay (PLA) in neuronal primary cultures, where it was observed that the expression of CB1R-NMDAR complexes was decreased upon alpha-synuclein treatment. These results point to a role of CB1R-NMDAR complexes as a new therapeutic target in Parkinson's disease.

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P.26. INTRANASAL IRBESARTAN DELIVERY: A PROMISING APPROACH TO FIGHT COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

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Clinical studies have demonstrated that angiotensin receptor blockers (ARBs) offer protection against Alzheimer's disease in hypertensive patients, suggesting their potential in treating or delaying disease progression. However, the blood-brain barrier (BBB) presents a significant obstacle to accessing the brain. Therefore, our aim was to assess if intranasal Irbesartan (IRB) could induce neuroprotective effects in an lipopolysaccharide (LPS)-induced neuroinflammation mice model. To overcome this barrier, IRB was administered IN to mice, enabling direct targeting of the brain. A comparative pharmacokinetic study was conducted in male CD-1 mice, administering IRB via intravenous (IV) and IN routes. IRB concentrations were measured up to 8 hours post-dosing in plasma, brain, and lungs. Efficacy was evaluated following IN administration of IRB (40 mg/kg, 10 days) to C57/BL6 male mice. On the final day, LPS was injected, and long-term memory was assessed using the novel object recognition test. Subsequently, animals were sacrificed for analysis of dendritic spines and neuroinflammation, neurodegeneration and cognitive function biomarkers. The results demonstrated that IN administration led to faster and more extensive delivery of IRB to the brain compared to the IV route ($p<0.0001$). Systemic absorption was slower but more prolonged after IN administration, while lung concentrations remained similar between routes. Notably, lung concentrations were statistically higher 15 minutes post-dose following IV injection ($p<0.001$). Efficacy studies revealed that IRB significantly ameliorated memory loss induced by LPS (discrimination index: -0.0995 vs 0.1813). This effect was associated with increased cortical dendritic spines ($p<0.05$), mRNA expression of Pi3kca ($p<0.01$), and Creb1 ($p<0.05$). IRB also stimulated the antioxidant defense system by elevating SOD2 ($p<0.01$) and Gpx-1 ($p<0.05$) protein levels, while decreasing LPS-induced astrogliosis ($p<0.05$), thereby preserving cognitive function. Overall, this study underscores the potential of IN administration of IRB to directly target the brain and mitigate LPS-induced cognitive decline by reducing neuroinflammation and activating neuroprotective pathways.

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P.27. MOLBOOLEAN STAINING REVEALS HIGH PROPORTION OF D2 RECEPTORS FORMING A2A-D2 HETEROMERS IN STRIATAL NEURONS OF MPTP-LESIONED PARKINSONIAN PRIMATES

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta, leading to striatal dopamine deficiency. The A2A and D2 receptors form heteromeric complexes in striatal neurons that play an important modulatory role in fine-tuning motor control.
The aim of this study was to assess, for the first time, the proportion of individual A2AR, of individual D2R and of A2AR-D2R heteromers (A2A-D2Het) present in a primate 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) PD model, with and without showing dyskinesias. This has been possible to achieve thanks to “MolBoolean” technique. MolBoolean is a novel immunoassay technology developed to quantify the relative proportions of monomers and dimers for a given pair of interacting proteins within cells and tissues.
Analyzing and comparing the levels of free A2A and D2 receptors, as well as A2AR-D2R heterocomplexes, in striatal neurons of this non-human primate model which closely resembles Parkinson's disease pathology in humans, provides critical insights into molecular alterations relevant to the pathogenesis and treatment of Parkinson's disease.

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P.28. NUT DIET ALLEVIATES COGNITIVE IMPAIRMENT IN AN APP/PS1 MURINE MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is characterized by cognitive decline, amyloid plaque deposition, and neurofibrillary tangles. Also, neuroinflammation and oxidative stress have a key role in its pathophysiology. Nuts provide a modulating effect in the antioxidant system which could lead to a prevention of cognitive impairment. Thus, the aim of this study is to evaluate the effect of nut diet in the prevention of AD and associated pathological process.
C57BL/6J (WT) and transgenic APPswe/PS1dE9 (APP) male mice fed with standard (CT) or nuts diet from their weaning, were divided into four groups (WT CT, APP CT, WT NUTS and APP NUTS). At 6-months-old, they underwent insulin and glucose tolerance tests (ITT/GTT). Additionally, behavioral tests were conducted to assess learning and memory capacity. Inflammation and memory-related markers were also examined.
Our results demonstrated a glucose tolerance disruption in APP mice which was not reverted due to nut-based diet. By contrast, APP animals did not show alterations in ITT caused by the genotype. Behavioral tests revealed an improvement in cognitive deficits, reducing anxiety-like behavior and ameliorating memory impairments in those AD mice fed with nuts. In accordance, this group also presented a higher number of dendritic spines compared to APP CT. This improvement correlated with a reduction in inflammation, as evidenced by decreased reactivity of microglia and astrocytes which was associated with a reduction of amyloid β plaques in the hippocampus.
Overall, this study demonstrates the potential of a nut diet to beneficially impact cognitive function in the context of AD.

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P.29. LESSONS LEARNED FROM LAFORA DISEASE: ROLE OF GLYCOGEN METABOLISM IN GABAERGIC NEURONS IN EPILEPSY

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Lafora disease is a rare metabolic neurodegenerative disorder characterized by the accumulation of abnormal glycogen aggregates in several tissues, including the brain. This condition, which affects
adolescents, manifests as a severe form of progressive myoclonic epilepsy, marked by relentless seizures and rapid neurodegeneration. The disease represents a significant medical challenge due to its intricate molecular mechanisms and the limited efficacy of available therapeutic interventions.
We previously demonstrated that glycogen aggregates in Lafora disease accumulate both in neurons and astrocytes, and that astrocytic aggregates induce neuroinflammation. Our current research has led to the identification of a previously unrecognized role of glycogen within GABAergic interneurons, key regulators of neuronal excitability and inhibition. By employing in vivo models with altered glycogen metabolism, we show that abnormal glycogen accumulation in these interneurons contributes to the onset and progression of epileptic seizures in affected individuals. Specifically, we have observed disruptions in GABAergic signaling pathways, resulting in dysregulated inhibitory neurotransmission and heightened neuronal excitability, culminating in seizure activity.
Our study not only deepens our understanding of the metabolic causes of epilepsy in Lafora disease but also highlights the potential therapeutic targets within the glycogen metabolism pathway. By elucidating the intricate metabolic interactions between glycogen and neuronal function, our findings pave the way for the development of targeted interventions aimed at restoring metabolic homeostasis and alleviating epilepsy symptoms in affected individuals. Importantly, these results might have implications for the etiology of epilepsy beyond Lafora disease.

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P.30. CLINICAL GRADE PRODUCTION OF LARGE-SCALE NEURAL PROGENITOR CELLS FOR HUNTINGTON'S DISEASE TREATMENT

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Regenerative medicine aims to revolutionize medical practice by offering cures for severe diseases. This is achieved through the utilization of human pluripotent stem cell (hPSC) lines, which undergo controlled differentiation before being transplanted into patients. A significant challenge lies in developing safe, robust, and controlled differentiation protocols, requiring the adaptation of research-only reagents to good manufacturing practice (GMP) standards.
Cell-based therapies for Huntington's disease focus on restoring neuronal connectivity and function by replacing the affected neurons, the Striatal Projection Neurons (SPNs). Here, we present a robust and reproducible GMP-compliant protocol for the maintenance and differentiation of hPSC into neural progenitor cells (NPC) suitable for clinical applications. We demonstrate the preservation of cell survival, morphology, pluripotent properties, and attachment rates of hESC throughout maintenance and scaleup steps. During the cryopreservation process, we demonstrate that using a controlled-rate freezer, as well as increasing feezed cell concentrations, enhances the cellular recovery rate. Furthermore, we also
show the lose of scaling efficiency when using larger flasks than T75. Upon differentiation, NPC exhibits the characteristic markers of SPNs and demonstrate functionality upon maturation. Stringent quality controls, including mycoplasma, endotoxins, sterility, karyotype, microsatellites, flow cytometry and gene expression profile are systematically implemented in both instances to ascertain the effectiveness and safety of the therapeutic product. Overall, we report the feasibility of derivation and differentiation of clinical-grade hPSC lines into NPC under GMP-compliant conditions.

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P.31. B-AMYLOID BUT NOT TAU ENHANCES FEAR AND ANXIETY IN NOVEL ALZHEIMER´S DISEASE TRANSGENIC MICE

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Progressive cognitive decline and neuropsychiatric symptoms are common clinical features of Alzheimer's disease (AD). Emotional disturbances, including anxiety and fear, occur very early during clinical AD, when individuals meet criteria for mild cognitive impairment. The mechanistic link between the classical cerebral disease pathological features, amyloid-β (Aβ) and tau, and amygdala-dependent emotional symptoms in AD is largely unclear. Here we show that anxiety and fear symptoms are associated with Aβ accumulation but not tau pathology in emotion-related brain regions of AD transgenic mice. By generating and analyzing littermate control, APP, Tau and APP/Tau Tg mice we demonstrate an age-dependent increase of Aβ and phospho-tau accumulation in the hippocampus and basolateral amygdala (BLA) of APP/Tau Tg mice. Both males and females APP and APP/Tau Tg mice, but not Tau Tg mice, displayed enhanced innate and conditioned fear symptoms and a deficiency in extinction fear memory consolidation coinciding with enhanced accumulation of Aβ in β-aminobutyric acid (GABA)ergic neurons of the BLA. These behavioral alterations occur in parallel with decreased activity of hippocampal neurons as assayed in APP/Tau; cfosp-EGFP reporter mice. Overall, these results suggest a novel pathogenic role of intraneuronal Aβ in GABAergic interneurons on anxiety and fear symptoms in AD. This study clarifies the relationship between amyloid and tau pathologies, and it provides a useful mouse model to delineate the neurobiological and pathological mechanisms underlying neuropsychiatric symptoms in AD.

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P.32. NANOGBA-TO-BRAIN STRATEGY AS A NOVEL PARKINSON'S DISEASE TREATMENT

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Parkinson´s Disease (PD) is the most common neurodegenerative movement disorder. To date, only symptomatic treatments are available that cannot halt the neurodegeneration. Therefore, there is an urgent need to develop new strategies aimed at targeting PD.
The GBA gene encodes for the lysosomal enzyme ß-glucocerebrosidase (GBA) and heterozygous mutations in this gene represent the most important genetic risk factor for PD. PD patients with GBA mutations present earlier and greater cognitive decline and increased risk of mortality, associated with accelerated α-synuclein (α-Syn) pathology. The restoration of GBA activity in cellular and animal models of PD has proven to be therapeutic.
Enzyme replacement therapy (ERT) with recombinant GBA protein is successfully administered to Gaucher's disease (GD) patients with biallelic mutations in the GBA gene; however, recombinant GBA cannot cross the blood brain barrier, making ERT ineffective for PD. Our team has developed a GBA nanoconjugate (nanoGBA) to deliver active GBA to the central nervous system (CNS) through intranasal administration.
Objectives and methodology: 1- Confirm the intracellular uptake of nanoGBA and the delivery to the lysosomes; 2- Study the restoration of GBA activity and its consequences in our in vitro PD-GBA neuronal model and in patient derived fibroblasts.; 3- Study the in-vivo delivery in a GBA mouse model.
Results and conclusions: We confirmed the internalization of the nanoGBA conjugate and the delivery of exogenous GBA to lysosomes. We began the characterization of the effects triggered by nanoGBA treatment, proving its impact on the restoration of GBA activity and the clearance of substrates. In vivo assays in a mutant GBA mouse model showed that intranasal administration successfully delivers the nanoGBA conjugate to the CNS, leading to the recovery of GBA activity and the reduction in the accumulation of substrates.

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P.33. EXPLORING MITOCHONDRIAL QUALITY CONTROL MECHANISMS IN ASTROCYTES IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited dominant neurodegenerative disorder that gradually impairs the patient's motor, cognitive and affective functions. This disorder is originated by a CAG repeat expansion in the gene encoding the Huntingtin protein. In HD, the brain and mainly the Medium Spiny Neurons (MSNs) within the striatum are the cells that degenerate the most. Among the alterations defined in neurons, dysfunction in mitochondrial quality control mechanisms (MQCM) have been described.
Though HD alterations have been mainly studied in neurons, HD patients and HD animal models exhibit astrogliosis. It has been reported that mHtt can disrupt several functions in astrocytes and could be promoting neuronal dysfunction by non-cell autonomous alterations. Nevertheless, there is still no information regarding MQCM in striatal astrocytes in HD. We propose that mHtt expression in striatal astrocytes might interfere with MQCM preventing astrocytes from performing their functions correctly. In this study we focused on mitophagy, the specific degradation of mitochondria in the R6/1 mouse model of HD. Mitophagy is carried out mainly by the PINK1-Parkin pathway. Following activation of this pathway, the protein p62 can bind to mitochondria and lastly induce the cleavage of LC3I to LC3II that will be followed by the engulfment of mitochondria and final degradation. We found that p62 protein levels are significantly increased in R6/1 astrocytes in primary cell cultures, nonetheless we do not see increased levels of LC3II, a marker of activation of autophagy. Also in astrocyte cultures, colocalization of p62 with mitochondrial marker TOMM20 is higher in the R6/1 model, indicating increased mitophagy. In isolated ACSA2+ astrocytes we find increased protein p62 levels at 12- and 20-week-old R6/1 mice whereas LC3 gene is significantly less expressed in 20-week-old mice astrocytes. All these results suggest that mitophagy pathway is stuck at p62 accumulation in mitochondria. Nevertheless, more studies should be done to confirm these findings.

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P.34. DYSREGULATION OF THE AUTOPHAGIC-LYSOSOMAL PATHWAY IN PARKINSON'S DISEASE ASSOCIATED TO GBA

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The GBA gene encodes ß-glucocerebrosidase (GBA), a lysosomal enzyme. Heterozygous mutations in this gene are the foremost genetic risk factors for Parkinson's Disease (PD). A well-established association exists between GBA alterations and α-synuclein pathology, where reduced GBA activity correlates with increased α-synuclein levels. Conversely, elevated α-synuclein levels hinder GBA activity, observed in both in vivo and in vitro models and GBA-PD patient samples. Loss of GBA activity is linked to dysfunctional autophagy–lysosome systems and subsequent reductions in autophagy-dependent α-synuclein turnover. Maintaining neuronal homeostasis relies significantly on lysosomal function. A substantial number of genes associated with PD are implicated in the autophagic/lysosomal system (ALS), either encoding lysosomal proteins or proteins involved in modulating these pathways. Recent studies reveal that over 50% of PD patients have at least one pathogenic mutation in a gene encoding a lysosomal-related protein, with GBA being the most frequent, present in 10-12% of the PD population (PD-GBA), underscoring the role of lysosomal dysfunction in PD etiology. Our investigations into the link between GBA loss and alpha-synuclein pathology encompass multiple approaches: Molecular Mechanism: We describe a new molecular mechanism elucidating how the initial loss of GCase activity leads to general lysosomal dysfunction and alterations in lysosomal lipid composition. These changes impair chaperon-mediated autophagy (CMA), fostering abnormal alpha-synuclein accumulation. Lysosomal Dysfunction Biomarkers: We optimized the quantification of novel lysosomal dysfunction parameters in various biological samples (serum and CSF). This effort aims to identify and validate the most effective biomarker(s) of lysosomal dysfunction in newly diagnosed PD patients. This stratification intends to enhance clinical management and treatment outcomes. Therapeutic Approach: Our team successfully developed a novel therapeutic strategy to restore GBA activity in the central nervous system (CNS), utilizing nanotechnology [a new GBA nanoconjugate (nanoGBA)] for delivering active GBA protein via intranasal administration.

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P.35. FAIM-L EXPRESSION RESTORATION IN THE HIPPOCAMPUS AMELIORATES COGNITIVE DYSFUNCTION AND SYNAPTIC LOSS IN A MOUSE MODEL OF TAU PATHOLOGY

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Tau hyperphosphorylation and aggregation are central events in tauopathies, including Alzheimer's disease (AD). Its aggregates forming NFTs are linked to neuronal death and cognitive dysfunction. We observed that levels of FAIM-L, a neuro-specific isoform of the Fas Apoptotic Inhibitory Molecule, are reduced in hippocampus of AD patients and mouse models. Interestingly, FAIM-L loss is specifically associated to Tau rather to beta amyloid pathology. We also detected that FAIM-L binds Tau and is able to modulate its ubiquitination, a posttranslational modification related to Tau dysfunction. These observations, together with the known role of FAIM-L in synaptic plasticity, suggest that FAIM-L loss could promote AD progression. To explore this hypothesis, we characterized FAIM-L loss along the disease progression in P301S (PS19) mouse model of tauopathy. FAIM-L decrease is detected at 6 months of age, together with pTau/Tau increase. Interestingly, these events occur before other pathological alterations related to neuropathology and cognitive loss, detected in 9 months-old mice. To test a possible FAIM-L therapeutic effect, we studied the functional implications of its restoration in the hippocampus of PS19 mice using an AAVs injection strategy. Results revealed that FAIM-L overexpression prevents certain aspects of learning and memory impairment. In our study, FAIM-L prevented associative memory impairment, improved discrimination capacity in the T-maze test, and ameliorated habituation deficits and first insights of hyperactivity. Our findings also show that FAIM-L restoration maintains synaptic integrity, preventing dendritic spine loss and PSD95 reduction. Our results confirm FAIM-L implication in AD linked to Tau pathology but also settle it as an essential element for neuronal survival, synaptic maintenance and cognition. These findings allow us to suggest that FAIM-L could confer early protection to Tau-associated alterations and place it as a potential therapeutic target for AD and possibly for FTDP-17 and other tauopathies, which needs to be further investigated.

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P.36. GPR37 PROCESSING AND EXPRESSION IN NEURODEGENERATION: A POTENTIAL MARKER FOR PARKINSON'S DISEASE PROGRESSION RATE

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The orphan G protein-coupled receptor 37 (GPR37), widely associated with Parkinson's disease (PD), undergoes proteolytic processing under physiological conditions. Its N-terminus is proteolyzed by a disintegrin and metalloproteinase 10 (ADAM-10), which generates various membrane receptor forms and the shedding of the ectodomain in the extracellular environment. Here, we investigate the processing and density of GPR37 in several neurodegenerative conditions, including Lewy body disease (LBD), multiple system atrophy (MSA), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and Alzheimer's disease (AD). An increase in receptor processing was observed exclusively in the early stages of LBD in both the PFC and the striatum, two key brain areas involved in neurodegeneration. In contrast, in MSA only the 52 kDa GPR37 specific form was shown in the striatum. This form was also elevated in the PFC and striatum of AD necropsies. On the contrary, the processing of GPR37 remained unaffected in the brain of patients with CBD and PSP. Furthermore, while the content of ecto-GPR37 increased in the CSF of PD patients, in MSA, CBD and PSP subjects the levels were not altered. Importantly, within patients with PD, those who showed rapid progression of the disease did not have elevated levels of ecto-GPR37 in the CSF, while those who slowly progressed showed a significant increase, suggesting a possible prognostic use of ecto-GPR37 in PD. This research underscores the distinctive processing and density patterns of GPR37 in neurodegenerative diseases, offering crucial insights into its potential role as a predictor of PD progression rates.

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P.37. FUNCTIONAL ANALYSIS OF NEURONAL ACTIVITY IN HUMAN BRAIN ORGANOIDS AS A MODEL OF TAUOPATHIES

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Spontaneous neuronal activity (SNA) plays an important role in the maturation and refinement of neuronal connections. However, neurodegenerative diseases, like tauopathies, can significantly alter SNA. Changes in SNA after the expression of tau mutations have not yet been analysed at presymptomatic stages. Furthermore, the currently available models lack the necessary complexity of the human brain to fully understand the underlying mechanisms of tau pathology and its effects on SNA. To address this, we present a novel in vitro platform utilizing cortical organoids (COs) derived from hPSCs - to unravel the effect of tau pathology on SNA by exploring the changes that occur after the inclusion of mutated and non-mutated tau. We have characterised COs using both commercial and healthy donor-derived hPSCs. To model tauopathy, COs were infected with adeno-associated virus that express either mutated P301L-tau or full-length non-mutated human tau (2N4R). We employed calcium imaging techniques using genetically encoded calcium indicators and two-photon microscopy recordings to analyse changes in SNA patterns and their correlation with tau pathology. Through overexpression of P301L-tau, we successfully developed COs that exhibit hyperphosphorylated tau as observed by biochemical analysis of phospho-tau (Ser422) and AT8, as well as tau aggregates observed by positive staining for thioflavin-S. Additionally, we have shown that these COs have SNA with observed changes in neuron firing frequency between P301L-tau, 2N4R-tau, and uninfected COs.
Our findings suggest that tau mutations alter SNA of COs and that the model can be used to further explore the functional consequences of tau mutation-mediated changes in SNA.

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P.38. CONTRIBUTION OF THE NEUROMELANIN-LINKED IMMUNE RESPONSE TO PARKINSON'S DISEASE PATHOGENESIS

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Activation of both innate and adaptive immune responses occurs in Parkinson's disease (PD) brains, and these PD-linked inflammatory changes are highly localized within neuromelanin (NM)-containing areas. However, the contribution to the neurodegenerative process of each branch of the immune response and its relationship with NM is still not clear. The fact that, in contrast to humans, NM is absent in common experimental animals such as rodents has historically hindered research in this area. For that reason, our group developed novel NM-producing PD rodent models based on the viral vector-mediated overexpression of melanin-producing enzyme tyrosinase (TYR) in the substantia nigra (SN). Here we used these models and human postmortem PD brains to characterize the relationship between the NM-linked immune response and PD-like pathology. To determine the relative contributions of the adaptive and innate compartments to this response, we used, respectively, genetically-modified MHC-II-deficient mice, which lack the capacity of MHCII-mediated antigen presentation, or pharmacological modulation of the innate response with ivermectin (IVM), a compound that induces an anti-inflammatory microglial phenotype.
In both human PD brains and NM-producing rodents, AI-based quantifications of the inflammatory/immune response confirmed increases in microglial/macrophage activation (Iba1/CD68), astrocytic response (GFAP) and T-cell infiltration (mostly CD8) in close association with extracellular NM released from dying neurons. In TYR-expressing rodents, both innate and adaptive immune reactions occurred at very early stages of the neurodegenerative process, even preceding overt neurodegeneration. We are now assessing the effects of modulating the innate (IVM treatment) or adaptive (MHC-II KO mice) immune responses on NM-linked neurodegeneration in these animals. Overall, early activation of immune responses by extracellular NM appears to contribute to PD pathology and progression, while the specific relevance of each immune compartment is currently under study.

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P.39. BIOFLUID-DERIVED EXTRACELLULAR SMALL RNAS AS PREMANIFEST BIOMARKERS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the Huntingtin gene. Molecular biomarkers categorizing mutation-carriers during the preclinical stage preceding the functional decline, can assist in optimizing patient management and potentially enable targeted interventions. Extracellular small RNAs (exRNAs), which can be found in body fluids as freely circulating, associated to protein-complexes, and/or encapsulated in extracellular vesicles (EVs), are a promising source of biomarkers since their expression levels are highly sensitive to pathobiological processes. Results from our group sugget that human plasma EV-sRNAs are genarally and early downregulated in mutation-carriers, and that this deregulation is associated with premanifest cognitive performance. Herein, we aimed to define exRNA-based biomarkers to monitor changes that occur in the pre-symptomatic stage, by exploring specific candidates in plasma and cerebrospinal fluid (CSF). The expression of seven candidate sRNAs (tRF-Glu-CTC, tRF-Gly-GCC, miR-451a, miR-21-5p, miR-26a-5p, miR-27a-3p, and let7a-5p) was validated by qRT-PCR showing a significant diagnostic accuracy at premanifest stage. Of these, miR-21-5p was significantly decreased over time in longitudinal samples; and miR-21-5p and miR-26a-5p levels correlated with cognitive changes in the premanifest group. Furthermore, specific plasma validated sRNAs were found to be significantly altered in the opposite direction in CSF samples of the same patients. In summary, the present results suggest that plasma and CSF exRNAs are early deregulated in HD. In addition, specific sRNAs reflect the premanifest progression and cognitive changes occurring before symptoms manifest. Overall, our results define a novel early RNA-based biosignature in HD with potential to improve HTT mutation-carriers classification.

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P.40. VESICULAR SMALL RNA-SECRETOME IS PERTURBED IN HUNTINGTON´S DISEASE NEURONS

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the Huntingtin gene (HTT). Despite that most studies have been historically concentrated on the pathogenic role of the resultant mutant HTT protein, growing insights on HD indicate that altered small non-coding RNA (sRNA) are involved in the pathophysiology. Data from our lab showed that sRNAs from HD patients´ brain are sufficient to induce neuronal toxicity and mediate neuroinflammation in vivo. In the paradigm of HD, these harmful sRNAs may be released in the extracellular space as freely circulating, associated to protein/lipid complexes, and/or encapsulated in extracellular vesicles (EVs), and could mediate paracrine toxicity. Herein, we used HD human embryonic stem cells (hESCs)-derived neuron cultures (neuron-hESCs) to elucidate the possible altered release of extracellular sRNAs (exRNAs) in HD. Using an optimized method for EVs purification from cell supernatants by Size-exclusion chromatography (SEC) and Ultrafiltration (UF), we explored the exRNA composition of the vesicular (EVs) and extravesicular (NonEVs) neuron-hESCs secretome through an exhaustive analysis pipeline of sRNA sequencing data in HD and Control neuron-hESCs lines. Characterization of hESCs-EVs revealed no differences in size and morphology of EVs between HD and Control. We showed heterogeneous proportions of exRNAs biotypes between intravesicular and extravesicular compartments and we highlighted that, inside EVs, different sRNA biotypes, such as miRNAs and tRNA fragments (tRFs), are significantly differentially expressed in HD compared to Control neuron-hESCs cell lines. Interestingly, specific miRNAs and tRFs species already described as deregulated in HD plasma-EVs, CSF and/or putamen samples, appeared similarly altered in this paradigm. These findings suggest that exRNAs release is altered in human HD neurons, and that these exRNAs may contribute to the paracrine toxicity observed in previous studies.

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P.41. CANNABINOID RECEPTOR TYPE 1 IN HIPPOCAMPAL ASTROCYTES OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative disease characterized by motor, cognitive and psychiatric symptoms. To date, drug treatment for this disease are mainly designed to ameliorate its involuntary movements although the pathology tends to debut earlier with considerable cognitive declines. In order to treat these memory disturbances, brain structures involved in memory and learning processes must be carefully investigated. Although the hippocampus remains a relatively unexplored brain structure in HD, hippocampal pathologies have been related to cognitive dysfunctions observed in both patients and mouse models of HD. Searching for new therapeutic scopes, cannabinoid receptor type 1 (CB1) is emerging as a potential target for cognitive dysfunction in HD. CB1 is one of the most expressed receptors in the hippocampus, contributing to the physiologic regulation of cognition and motor behavior. In particular, hippocampal expression of CB1 in astrocytes leads to a cascade of calcium mobilization and glutamate stimulation that contributes to normal memory functions. Interestingly, CB1 is also altered in HD patients and mouse models. Preliminary results from the lab showed that CB1 is decreased in the R6/1 mouse model of HD and this alteration can be linked to short and long-term memory deficits. Considering that viral CB1 increase in hippocampal astrocytes is an approach capable of restoring memory functions, the aim of this project is to characterize hippocampal CB1 in astrocytes and the role that this receptor plays in calcium homeostasis as a potential target for cognitive dysfunction in HD.

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P.42. LONGITUDINAL CORTEX-DEPENDENT MOLECULAR AND BEHAVIORAL ALTERATIONS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative condition marked by a repeat expansion of CAG within the HTT gene's exon 1, leading to motor, cognitive, and psychiatric issues. HD pathology manifests as a progressive disruption of the cortico-striatal pathway preceding significant motor symptoms. The R6/1 mouse model mirrors disease progression, exhibiting molecular and behavioral abnormalities, with cognitive deficits preceding motor impairments, occurring at 12 and 16 weeks, respectively. Building on our prior findings indicating reduced functional connectivity in secondary motor cortex (M2) circuitry via rs-fMRI in HD mice, we aimed to investigate the onset of cortical-associated behavioral and molecular alterations in the R6/1 mouse model of HD from 4 to 16 weeks of age. At the behavioral level, we evaluated somatosensory and fine motor function, related to sensory and motor cortices, through the adhesive removal test (ART), and we found fine motor deficits emerging at 8 weeks. Notably, ART results hint at potential benefits of early training, with differences diminishing by 12 weeks post 4 weeks of testing. Also, anxiety and compulsive behavior, linked to orbitofrontal cortex, was assessed via the marble burying test (MBT). Intriguingly, R6/1 mice exhibit reduced marble burying from 8 weeks, suggestive of apathetic/depressive behaviors akin to human patients. At the molecular level, we are exploring alterations in cAMP-PKA signalling pathways, involved in synaptic plasticity and known to be disrupted in HD. We conducted Western blot analysis using samples from the frontal motor cortex, somatosensory cortex, and striatum of wild-type (WT) of 8, 12, 16, and 20 weeks old mice. Particularly, we focused on pPKA (phosphorylated protein kinase A), PKA alpha, and pPKA substrates. These combined findings unveil motor and psychiatric symptoms linked to distinct cortico-striatal impairments in early HD stages, preceding motor coordination and learning deficits, aiding in disease detection and monitoring.

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P.43. CANNABINOID COMPOUND AS THERAPEUTIC SCOPE FOR DEPRESSION SYMPTOMS IN HUNTINGTON'S DISEASE

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Huntington's Disease is a neurodegenerative disorder caused by a triplet elongation in huntingtin gene, causing the characteristic chorea spasm, cognitive and psychiatric symptoms. HD affects up to 5 people per 100.000 and depression is one of the most prevalent psychiatric symptoms.
Up to now, no specific therapies are available to ameliorate psychiatric symptoms, revealing the lack of knowledges. Searching for new strategies, cannabinoid receptor 1 (CB1) is emerged as a new potential target for depressive symptoms since is one of the most expressed receptor in the brain and has been involved in cognitive and psychiatric phenotypes.
Preliminary studies in the lab showed that CB1 is decreased in the hippocampus of HD mouse model and this alteration is linked to memory dysfunctions. CB1 agonist WIN 55,212-5 ameliorates short and long-term memories in HD mouse model, without effects in controls. The current work aims to explore CB1 alterations in brain regions related to depressive symptoms and investigate its role in depression. In particular, we want to characterize CB1 in the prefrontal cortex and hippocampus at different time points and in both sexes and assess cannabinoids contributions in depressive symptoms. Interestingly, our results showed that cannabinoids can rescue depression and prevent cognitive symptoms in early stages of the disease. In conclusion, CB1 and CB1-based drug emerged to be potential both for depressive and for memory deficits.

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P.44. CONTRIBUTION OF SMALL RNAS IN THE INFLAMMATORY PROCESSES OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) pathogenesis, marked by severe neuronal death in the striatum, is incredibly complex and involves many molecular mechanisms. Glial activation and neuroinflammation are prominent features in HD, although the drivers of immune activation and their effect on HD progression remain unclear. Small RNAs (sRNAs, <200 nucleotides) are early dysregulated in HD brains. Our previous data showed that sRNAs from HD patients' brains (HD-sRNAs) are sufficient to cause neurotoxicity and neuroinflammation in vivo. Recent studies indicate that endogenous RNAs can trigger immune pathways through specific sensing machinery. Here, we used multiple approaches to study RNA sensors and their downstream immune factors in the context of HD. First, we determined the expression of RNA sensors and inflammatory cytokines in naïve mice 24h after being injected with sRNA from HD or control (CTL) brains, or a vehicle substance. Our results show a significant increase in inflammatory cytokines, and several RNA receptors, in animals injected with HD-sRNA compared to CTL-sRNAs, indicating an acute inflammatory response specific to HD sRNAs. When we assessed their levels in symptomatic R6/1 and wild-type mice, we observed a milder inflammatory state in R6/1 with few sensors activated. Then, we measured the expression of these genes in brains from HD and healthy individuals, revealing most cytokines significantly overexpressed in HD patients' brains, and all tested RNA sensors were robustly activated in HD cases. These results suggests that HD pathogenesis in humans has a stronger inflammatory component than in animal models. Lastly, we examined neurons and astrocytes derived from HD human embryonic stem cells, which have similar expression patterns to those in patients' brains. Overall, our results point to RNA-mediated inflammation as an important contributor to HD pathogenesis. Future investigations will provide further insights on the characteristics of these immunogenic HD-sRNAs and the RNA sensors' specific role in HD.

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P.45. EARLY DISRUPTION OF HIPPOCAMPAL PARVALBUMIN INTERNEURONS CORRELATES WITH MEMORY DEFICITS IN ALZHEIMER'S DISEASE MICE

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Excitatory/inhibitory neurotransmission imbalance in memory neural circuits has been proposed to underlie memory deficits in Alzheimer's disease (AD). However, the cellular mechanisms by which these neuropathological hallmarks induce dysfunction of excitatory and inhibitory hippocampal neurons remain poorly understood. Here, we combined behavioral, molecular, biochemical, cell type-specific RNA-seq, as well as conventional and advanced histological and microscopy analyses, including tissue clearing and expansion microscopy, to assess the effect of Aβ and tau accumulation in the hippocampus of male and female of double APP/Tau transgenic mice.
Our results show Aβ and tau pathologies in hippocampal excitatory neurons of 6-month-old APP/Tau mice, as well as tau accumulation at hippocampal synapses and reduction of synaptic proteins, coinciding with early sex-dependent spatial learning deficits. Interestingly, APP/Tau mice at 6 months of age show region-specific loss, morphological changes and altered expression of synaptic genes in hippocampal parvalbumin (Pvalb)-positive interneurons, despite absence of Aβ and tau accumulation in these cells. Taken together, our results suggest that early transcriptional and structural changes in Pvalb-positive interneurons in response to accumulation of Aβ and tau pathologies in excitatory neurons may contribute to memory loss in APP/Tau mice.

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P.46. STUDYING THE ROLE OF CIRCADIAN DESYNCHRONISATION IN THE MICROBIOTA-GUT-BRAIN AXIS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a devastating neurodegenerative process. AD pathology is accompanied by severe cognitive dysfunctions. Altered circadian clock-controlled behavioural processes such as a disturbed sleep–wake cycle, as well as functional deterioration in the Suprachiasmatic nucleus (SCN), home of the central clock, have also been associated with AD. Decline of central clock function in the SCN of AD patients and alterations in the signalling pathways downstream of the SCN likely result in a weakening of systemic circadian clock network integrity.
AD is increasingly perceived to not only affect the brain, but also to impact on peripheral tissue physiology. In turn, peripheral tissues modulate the pathology in the brain. This is particularly true for the intestine and its role in the microbiota – gut – brain axis. AD patients develop dysbiosis at early stages of the disease and transplantation of AD-related microbiota propagates AD-like symptoms to healthy recipients, suggesting that the microbiota contributes to the development of AD pathology. The gut microbiota and the circadian clock network are inextricably intertwined. Abundance, location and composition of the intestinal microbiota follows a circadian rhythm which is depending on the host circadian clock network. Inversely, the microbiota influences circadian rhythms locally and in distal tissues. How the microbiota is implicated mechanistically in promoting AD pathology and whether deteriorating circadian rhythmicity in the microbiota – gut – brain axis contributes to the pathogenesis is only poorly understood.
Here we are providing first insights into the diurnal timing in the microbiota – gut – brain axis in AD and discuss how daily cues from the gut might regulate AD pathology.

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P.47. NEW NEURODEVELOPMENTAL PATHOLOGY DUE TO A DEFECT IN CELL TRAFFICKING: VPS8 DEFICIENCY

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The number of diseases associated to alterations in cell trafficking has increased over 7 folds in the last 10 years, outstanding now as the major neurometabolic group of diseases. Moreover, defects in cell trafficking underly not only in neurodevelopmental diseases but also in more frequent ones such as Parkinson or Alzheimer disease. Monogenic defects on cell trafficking represent an unbeatable frame for the study of these processes homeostasis and are key to define therapeutic alternatives for their treatment.
In this work we have characterized the alterations in membrane trafficking in a patient bearing two heterozygous mutations in VPS8. The patient presented with a neurological phenotype of Pediatric Parkinsonism, including neurodevelopmental delay, epileptic encephalopathy and intellectual delay. Since no mutation in this gene had previously been described, we performed a personalized study of the disease to confirm the diagnosis and to find new therapeutic strategies for the patient.
VPS8 is part of the class C core vacuole/endosome tethering (CORVET) complex, which mediates the formation of the early endosomes, formed upon internalization of a cargo through endocytosis. Moreover, VPS8 is also involved in the recycling routes, avoiding internalized proteins degradation. In order to characterize the endosome subpopulations in patient and controls primary fibroblasts we analyzed Rab effector protein levels as surrogate markers of each cluster. According to our results, the patient fibroblasts suffered a delay in endosomal maturation, along with an unbalance in the recycling routes.
In summary, we have described the first ever patient bearing mutations in VPS8, which result in a neurodevelopmental phenotype of Pediatric Parkinsonism. Understanding the pathophysiology of this disorder will pave the path for the discovery of new therapeutic strategies not only for itself, but for other diseases such as Parkinson or autophagy-related disorders.

P.48. VPS13A KNOCKDOWN DEREGULATES DIACYLGLYCEROL METABOLISM AND SIGNALING IN A MOUSE MODEL OF CHOREA-ACANTHOCYTOSIS

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Chorea acanthocytosis (ChAc) is an ultra-rare inherited neurodegenerative disease, caused by a VPS13A gene mutation provoking the lack of expression for the VPS13A protein. The main pathophysiological feature of ChAc patients is a progressive dysfunction of the corticostriatal pathway. VPS13A acts as a bulk lipid transporter between different organelles, thus it has been proposed to act as a lipid transfer protein. However, the precise role of VPS13A in lipid function in neurons remains unknown. To elucidate this role of VPS13A in the lipid profile, we established a shRNA-based Knockdown (KD) mouse model and analyzed the lipidomic profile of corticostriatal brain samples by liquid chromatography-high resolution mass spectrometry (LC-HRMS). We studied the concentration of glycerophospholipid and sphingophospholipid species, and some of their precursors and derivates in KD and Control (Ctrl) mice brain tissue. In our targeted lipidomic analysis, we detected 172 lipid species and, after the construction of a regression model and a complementary partial least squares-discriminant analysis (PLS-DA) and the subsequent estimation of the variable importance of projection (VIP) scores, we found significantly elevated levels of 14 different species of diacylglycerol (DAG) in KD brain tissue, compared to Ctrl. GO enrichment analysis resulted in confirmation of DAG acting as a second messenger, activating multiple signaling cascades with the affectation of the synthesis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). To validate the results, we are currently investigating the potential changes in various enzymes associated with DAG-related pathways. We are analyzing the levels of DDHD2, CHPT1, CEPT1, and the phosphorylation levels of PKC by western blot, in control and VPS13A-KD neuronal cultures, to further establish if the elevated DAG levels affect intracellular pathways. Overall, our lipid profiling study could offer new insights into the role of VPS13A in neurons and improve our comprehension of ChAc pathophysiology.

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P.49. IMPACT OF VPS13A IN NEURONAL MITOCHONDRIAL HOMEOSTASIS IN A MODEL OF CHOREA-ACANTHOCYTOSIS

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Loss-of-function mutations in the human vacuolar protein sorting 13 homolog A (VPS13A) gene leading to depletion of VPS13A protein are causative for Chorea Acanthocytosis (ChAc), an ultra-rare disease, characterized by neurodegeneration mainly affecting the basal ganglia. ChAc patients present movement disorders such as chorea and dystonia, but only symptomatic treatment is currently available. In neurons, VPS13A is located in soma and neurites and colocalizes with endoplasmic reticulum and mitochondria organelles. VPS13A knockout is described to alter mitochondrial fragmentation in cell line models. This study aimed to understand the impact of VPS13A on mitochondrial functioning in neurons. We first evaluated VPS13A presence in mitochondrial-associated membranes (MAMs) and assessed VPS13A interacting partners in the wild-type mouse brain using a specific protein immunoprecipitation followed by mass spectrometry to understand its function. We found that VPS13A is located specifically in MAMs and that interacts predominantly with mitochondrial metabolism-related proteins. Then, to analyze the role of VPS13A in mitochondrial homeostasis, we used a shRNA-based VPS13A knockdown (KD) model on neuronal cultures. We found VPS13A downregulation induced an excessive production of superoxide species in the mitochondria measured by MitoSOXTM. Moreover, we show a decrease on mitochondrial size located in the neurites of KD neurons. Additionally, VPS13A KD led to a decrease in membrane potential depolarization response in the presence of an oxidative phosphorylation uncoupler. To further investigate the mechanism of a possible mitochondrial dysfunction, we used the Mito Stress Test Kit from Seahorse Analytics. Our findings demonstrate that VPS13A KD in neurons results in a decrease in the basal oxygen consumption rate and also a decrease in the extracellular acidification rate. Taken together, our results show that VPS13A KD induces mitochondrial stress and energy metabolism alterations, highlighting the key role of VPS13A in mitochondrial homeostasis and functioning, which contribute to unveiling the pathophysiological mechanisms of ChAc.

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P.50. IDENTIFICATION OF CIRCULATING MICRORNAS RELATED TO THE COGNITIVE IMPAIRMENT STATUS IN POST-COVID-19 PATIENTS

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Post-COVID-19 condition (PCC) develops after the acute phase of the SARS-CoV-2 infection with cognitive symptoms in 10-40% of cases, including executive, memory and mood disorders. MicroRNAs (miRNAs) are vital neurophysiological regulators and potential biomarkers in neurodegeneration. This study seeks to uncover functional pathways of circulating miRNAs differentially expressed in PCC patients with cognitive impairment and their associations with scores of a neuropsychological battery.

A complete neuropsychological battery assessed memory, executive function, processing speed, and depressive symptomatology in 25 CCP patients and 10 healthy controls. PCC subjects were divided into two groups: cognitive impairment (PCC-CI) for scores <1.5 standard deviations below the norm and MoCA <= 24 (n = 10), and cognitively healthy (PCC-CH) for scores within normal ranges in all domains (n = 15). Plasma miRNAs were quantified using a TaqMan OpenArray (Applied Biosystems) and functional analysis of differentially expressed miRNAs was performed using prediction tools accessible from DIANA mirPath v4. Associations were estimated using the Spearman correlation test. Six (miR-214, miR-301b, miR-362, miR-455, miR-483 and miR-96) and two (miR-195 and miR-224) miRNAs were significantly downregulated and upregulated in PCC-CI patients compared to PCC-CH patients, respectively. Functional analysis revealed that they are associated with FOXO and Hippo signaling, protein processing in endoplasmic reticulum and cellular senescence KEGG pathways, which have been described to be involved in Alzheimer's disease, aging and longevity in humans. Correlation analysis showed a direct correlation between miR-483 and verbal memory variables and an inverse correlation with depression. miR-455 levels were directly correlated with measures of social cognition.

This study reveals a subset of eight miRNAs differentially expressed in PCC-CI patients, which are interesting on a functional basis. Among them, miR-483 and miR-455 levels showed correlations with cognitive status. Further validation is required expanding the sample size.

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P.51. CONTRIBUTION OF CATECHOLAMINE OXIDATION TO PARKINSON'S DISEASE NEURODEGENERATION

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In Parkinson's disease (PD) there is a preferential degeneration of neurons that contain the pigment neuromelanin, especially dopamine-producing neurons of the substantia nigra (SN). Neuromelanin is an oxidative byproduct of dopamine metabolism that progressively accumulates with age and is synthesized, at least partially, by oxidation of excess cytosolic tyrosine, L-DOPA or dopamine into [o-]quinones, which then convert into eumelanin or pheomelanin melanic components via the formation of aminochrome or 5-S-cysteinyl-dopamine/dopa precursors, respectively. In contrast to humans, neuromelanin does not appear spontaneously in most animal species, including rodents, and PD is an exclusively human condition. Using novel humanized neuromelanin-producing rodent models based on the constitutive or viral vector-mediated overexpression of melanin-producing enzyme tyrosinase, we recently found that neuromelanin can trigger PD pathology when accumulated above a specific pathogenic threshold. Because dopamine oxidative species that act as neuromelanin precursors have been reported to be toxic in vitro, here we assessed whether catecholamine oxidation per se may directly contribute to neuromelanin-linked neurodegeneration in vivo. In the SN of both PD brains and neuromelanin-producing rodents, we found that dopamine oxidation was similarly increased compared to controls. However, these increased dopamine oxidative species were not apparently toxic per se in vivo, as seen in wild-type mice chronically treated with L-DOPA or in naïve rats intranigrally injected with oxidized neuromelanin precursors. In contrast, reduction of intracellular neuromelanin levels by decreasing dopamine oxidation into neuromelanin with antioxidant compounds, including cerium oxide nanoparticles, resulted in a significant attenuation of neurodegeneration, both in vitro and in vivo. Our results indicate that, while PD-like neurodegeneration linked to neuromelanin accumulation is not directly caused by catechol oxidative species, age-dependent neuromelanin levels can be therapeutically modulated in vivo by targeting catechol oxidation, thereby opening a new potential therapeutic path for PD.

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P.52. EFFECTS OF B-CARYOPHYLLENE IN THE APPSWE/PS1DE9 MOUSE MODEL OF FAMILIAL ALZHEIMER'S DISEASE: MOLECULAR AND BEHAVIORAL EVALUATION

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Alzheimer's disease (AD) is a neurodegenerative and progressive type of dementia, characterized by beta-amyloid plaques, glial responses, neuronal death and synaptic loss that leaves to neuroinflammation and cognitive impairment. β-caryophyllene (BCP) is a sesquiterpene found in various plant essential oils, known for its pharmacological activities such as antioxidant, anti-inflammatory and immune-modulatory, suggesting that it could be a promising therapeutic target. Thus, the aim of this study is to evaluate whether BCP administration in familial AD mice model is able to inhibit the main mechanisms related to the pathophysiology of the disease.
APPswe/PS1dE9 (APP) double transgenic and wild type C57BL6/J (WT) female mice (5-month-old) were treated 3 times a week for 4 weeks with BCP (48 mg/kg) or vehicle (VEH) intraperitoneally. After, animals were subjected to morris water maze (MWM) and novel object recognition test (NORT) to evaluate cognition, open field (OF) to asses anxiety behavior. Moreover, the assessment of hallmarks such as Aβ42 and dendritic spines were performed.
Our data show a significant improvement in spatial learning and memory in those APP mice treated with BCP in comparison to APP VEH, reaching similar value to WT, evidenced by the MWM and NORT. These cognitive changes correlate with a reduction in dendritic spine loss in the hippocampus. In this line, the same pattern in anxiety-like behavior was observed. APP BCP showed a significant reduction of anxiety in comparison to APP VEH. Finally, no significant differences were observed in Aβ42 levels in cortex when comparing both transgenic groups.
In conclusion, our findings demonstrate that treatment with BCP enhances cognition, reduces anxiety-like behavior and preserved dendritic spine density, protecting against this pathological mechanism. However, BCP did not significantly affect the Aβ42 levels of transgenic mice cortex. These results highlight the potential neuroprotective benefits of BCP, which could contribute positively to stop AD development.

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P.53. DELTA9-TETRAHYDROCANNABINOL AND CANNABIDIOL MODULATE HIPPOCAMPAL GLUTAMATE DYNAMICS IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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The endocannabinoid system (ECS) has gained attention as a potential target to treat Alzheimer's disease (AD) due to its neuroprotective, antioxidant, and anti-inflammatory properties. Consequently, natural cannabinoids like delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have emerged as potential drugs against AD. Previous findings from our research group demonstrated that a combination of non-psychoactive doses of THC and CBD mitigates cognitive decline in an AD murine model, the APP/PS1 mice. Cognitive decline in AD is linked to the dysregulation of glutamate transmission within the hippocampus. In this sense, we observed by in vivo microdialysis techniques that chronic THC+CBD treatment reduces excessive hippocampal glutamate levels in APP/PS1 animals. However, in vivo microdialysis has limited temporal resolution, constraining in-depth study of cannabinoid effects at the molecular level. To overcome these limitations, in the present study we set up fiber photometry techniques to evaluate the impact of THC and CBD on glutamate dynamics and neuronal activity in the hippocampus of APP/PS1 mice with a millisecond resolution. Using fluorescent biosensors, we evaluated acute effects of THC, CBD, or THC+CBD on glutamate release and neuronal activity (i.e. cytosolic calcium levels) in response to hippocampal electrical stimulation in anesthetized WT and APP/PS1 animals. We found that THC induces a higher glutamate release only in APP/PS1 animals when stimulating at high frequencies, while its combination with CBD reverts this effect. Besides, neuronal activation is reduced when both cannabinoids are present, revealing a synergistic effect of THC and CBD that could be of interest within this context of hyperexcitability.

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P.54. ROLE OF ALLELIC VARIANTS IN HLA GENES AS MODIFYING FACTORS FOR AGE OF ONSET IN AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by the progressive loss of motor neurons. Understanding the factors influencing the disease, including genetics factors such as HLA variants, may offer valuable insights into ALS pathogenesis and guide the development of personalized therapeutic approaches.
Objective: This study aimed to investigate the role of genetics variants from 10 major HLA genes in modifying the age of onset in a Spanish cohort of ALS patients.
Methodology: A cohort of 272 Spanish ALS patients was recruited, and their genomic DNA was purified. Patients with extreme phenotypes for age-at-onset were genotyped using a high resolution next-generation sequencing (NGS)–based HLA genotyping assay for HLA genes A, B, C, E, G, DRB1, DQB1, DQA1, DPB1, DPA1. Genetic association was analysed using BIGDAWG R package.
Results: Patients with extreme phenotypes for age-at-onset were identified: 33 early onset (<41 years) and 67 very late onset (>70 years). Patients with early age-at-onset displayed higher survival rates compared to those with very late age-at-onset. These patients were genotyped for 10 HLA genes as potential modifying risk factors for early age-at-onset ALS. A total of 254 HLA alleles were identified in the HLA genes analysed. Our BIGDAWG results revealed associations between specific HLA alleles and ALS phenotypes. After applying multiple-test correction, we identified statistically significant associations between alleles in the genes HLA-B, DPB1, DQB1, and the age of onset in ALS patients, utilizing an analysis of extreme phenotypes.
Conclusions: This study highlights the potential role of HLA alleles in influencing the age of onset in ALS. Alleles in the genes HLA B, DPB1, DQB1 could be risk factor for modifying the age of onset in ALS Spanish patients. Further research is required to validate these preliminary findings in larger patient cohorts.

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P.55. GENETIC ASSOCIATION STUDY OF VARIANTS IN GENES RELATED TO MG AND PD IN A COHORT OF PATIENTS WITH MG+PD FROM AN IBERIAN POPULATION

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In patients with both Myasthenia Gravis (MG) and Parkinson's Disease (PD), there may be a common genetic background for both diseases. In these patients, common genetic variants (MAF>5%) may be associated with susceptibility, or symptom's course, to both diseases. Objective: to evaluate whether common genetic variation might contribute to MG+PD risk, or as modifying factors, and whether that genetic risk might be enriched in our 19 case series compared with 107 control subjects from Iberian population. Methodology: 42 SNPs in 12 genes related to PD, MG, and myasthenic syndromes with an allele frequency in the Iberian population >5% were selected. The allele and genotype distribution of these SNPs were compared between MG+PD patients and control subjects by logistic regression using SNPassoc, using five different inheritance models (dominant, recessive, additive, codominant, overdominant). A comparison of the genetic and clinical risk factors (sex and age at disease onset) for susceptibility to MG+PD was carried out by multivariate logistic regression analysis between MG+PD patients and control subjects. Odds ratio with 95% confidence interval was calculated, multiple test correction was applied for statistical significance (p-value<0.0217). Results: the study found that 10 variants could act as risk factors for the MG+PD phenotype under the most significant models in the genes IL-1B, PTPN2, HLA-DQB1. SNPs in HLA-DQB1 were only detected when gender-based analysis was performed in male individuals. The study observed that variants in IL-1B might be associated with an earlier onset of MG, and a total of 7 genetic variants would be associated with the age at PD onset. Conclusion: Common genetic variants in autoimmunity-related genes (IL-1B, PTPN2, HLA-DQB1) could act as risk factors and phenotype modifiers in MG+PD patients.

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P.56. ASSESSING THE ROLE OF MITOCHONDRIAL DSRNA AS A TRIGGER FOR NEUROINFLAMMATION IN A MOUSE MODEL OF LEIGH SYNDROME

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Mitochondrial dysfunction has been classically associated with detrimental effects to cells that can lead to primary mitochondrial diseases (MD), which are known to be severe and usually fatal pathologies. Although the underlying mechanisms responsible for the neuronal death in these pathologies still remain unrevealed, there is compelling evidence that the release into the cytosol of either mitochondrial DNA (mtDNA) or mitochondrial double-stranded RNA (mtdsRNA) induce pathogenic inflammatory responses. In the same vein, previous results of our group indicate that the lack of the mitochondrial complex I subunit NDUFS4 in GABAergic neurons of the mouse olfactory bulb (OB) elicits a robust antiviral immune response and a noteworthy increase in the expression of cellular dsRNA sensors, as well as an increased presence of mtdsRNA associated to the dsRNA sensor PKR. Moreover, we have found that silencing the antiviral protein kinase R (PKR) expression decreases inflammatory markers in the affected brain areas of Ndufs4KO mice, suggesting a contribution to the disease progression by exacerbating the neuroimmune response. To dissect the contribution of mtdsRNA signaling to the antiviral response in the mouse model lacking Ndufs4 (Ndufs4KO), we have established a novel viral vector approach to achieve cell-type specific dsRNA degradation in GABAergic neurons, given that secreted brain RNAses play a key role in the degradation of intracellular and extracellular RNA. With the specific aim of efficiently reduce mtdsRNA cytosolic actions, in a cell-type specific manner, we have generated an AAV vector expressing dsRNAse4 in a Cre-dependent manner, that has been subsequently injected in the olfactory bulb of Gad2-Cre,Ndufs4KO and control mice, as it is one of the most affected GABAergic brain regions in this mouse model. Targeted degradation of mtdsRNA has been first explored in vitro in HeLa cell cultures and the olfactory bulb of dsRNAse4-injected mice compared to their respective control group. In addition, the neuroinflamatory status of this afected brain area has been assesed after dsRNase4 expression. Thus, our data suggest that targeted degradation of dsmtRNA may inhibit mitochondrial dysfunctioninduced antiviral responses, thus presenting a potential avenue for the treatment of MD.

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P.57. LICOCHALCONE A PROTECTS AGAINST HFD-INDUCED NEURODEGENERATION TROUGHT BRAIN AND LIVER MODULATION

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Neurodegenerative diseases approach is changing towards a systemic failure, focusing on the relevance of liver alterations altogether with brain dysfunction where metabolism and inflammation play an important role. Particularly, Licochalcone-A (LCA) has been described as an antidiabetic and anti-inflammatory drug. Therefore, this study aims to determinate the effect of LCA against neurodegeneration induced by metabolic syndrome.
To perform this study, C57BL/6J male mice were fed with control (CT) or High-Fat Diet (HFD) from weaning until the end of the experiment at nine-month-old. Subsequently, at eight-month-old, animals were intraperitoneally treated with 15 mg/kg/day of LCA or saline solution three times per week for 4 weeks. The experimental groups were designated as CT-Saline, HFD-Saline and HFD-LCA. Afterwards, behavioural assesments and Glucose/Insulin Tolerance tests were performed. Finally, hallmarks of synapsis and neuroinflammation were evaluated in the hippocampus, as well as, signs of damage on the liver.
The findings of this study demonstrated that LCA treatment succesfully prevented against brain alterations present after a chronic intake of HFD. Those enhacements included, protection against memory loss in the behavioural tests, mantainance of dendritic spine density, and mitigatation of neuroinflammatory processes such us microglial activation in the hippocampus. Furthermore, the administration of LCA reported an amelioration of metabolic disruptions caused by HFD, enhacing glucose metabolism and reducing insulin resistance, as well as decreasing liver weight and its histopathological hallmarks of damage.
In conclusion, these results suggest LCA as a potential treatment for neurodegenerative disorders, enhancing cognitive resilience and modulating key features such as metabolism and inflammation in both brain and liver.

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P.58. ZAC1 REGULATES GENES INVOLVED IN MULTIPLE SCLEROSIS

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Zac1, a zinc finger protein that regulates both apoptosis and cell cycle arrest, is an imprinted gene that regulates pro-neuronal genes during proliferation and differentiation of neural stem cells. Neurogenesis and gliogenesis are important cellular processes in the regeneration of the central nervous system after injury or damage. However, the role of zac1 in neurodegenerative diseases has been poorly studied. Multiple sclerosis (MS) is an inflammatory and demyelinating autoimmune disease of the central nervous system (CNS), whose inflammatory response is mediated by the activation of glial cells (astrocytes and microglia), which contribute to oligodendrocyte damage, demyelination, and axonal damage, compromising neuronal signalling. The objective of this work is to determine the role of Zac1 during demyelination. The expression of Zac1 has been determined in the experimental autoimmune encephalomyelitis (EAE), a common animal model of multiple sclerosis (MS), thus in human samples of MS patients. The expression of Zac1 is altered in both models. In EAE, the medullary expression of Zac1 is altered in the asymptomatic phase. Thus, Zac1 expression is upregulated in the cervical region ($p<0.001$) and downregulated in the thoracic region ($p<0.001$). In the symptomatic phase, Zac1 expression is downregulated at 14 days post-immunization (DPI) in all spinal cord regions ($p<0.001$), at 21 DPI in cervical regions ($p<0.001$). 01) and thoracic ($p<0.001$), and at 28 DPI only in the thoracic region ($p<0.01$). Zac1 is also downregulated in MS samples ($p<0,01$). Zac1 regulates the expression of genes involved in MS, since Zac1 binds to the promoters of the inflammatory genes NR1H3 and HLA-C in healthy patients but its binding is blocked in MS patients. Zac1 expression is altered in multiple sclerosis, and Zac1 regulates inflammatory genes involved in MS. In conclusion, Zac1 is a transcription factor involved in cellular neurodegeneration processes associated with cellular inflammation (glial activation) in MS.

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P.59. NEURONAL RTP801 AFFECTS ADULT HIPPOCAMPAL NEUROGENESIS IN VIVO IN HEALTH AND ALZHEIMER'S DISEASE

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Neurogenesis is the process of new neuron formation in the nervous system and it is maintained throughout life in specific neurogenic niches. Among them, the dentate gyrus (DG) of the hippocampus is gaining attention given the role that adult hippocampal neurogenesis (AHN) has in cognition and memory. RTP801/REDD1 is a stress-induced protein that inhibits mTOR signaling pathway. In addition, RTP801 has been linked to embryonic neurogenesis. Selective silencing of RTP801 in radial glia, by in utero electroporation, impairs migration and promotes premature differentiation of neural progenitors during cortex development. Moreover, neuronal RTP801 regulates neuroinflammation in Alzheimer's disease (AD) and selective silencing of its expression recovers gliosis hallmarks and neuroinflammation in the 5xFAD murine model of AD. Neuroinflammation is known to be a key regulator of AHN which dramatically halts the neurogenic process. To study the role of neuronal RTP801 in AHN in physiological conditions and in the 5xFAD mouse model of AD. In this study, 6-month-old male WT and 5xFAD mice were subjected to 1µL bilateral injections of rAAV2/8-H1-shControl-RSV-GFP (shCt) or rAAV2/8-H1shRTP801-RSV-GFP ((shRTP801) at CA1 and DG (mm). After 4 weeks animals were euthanized, and samples were processed for immunohistochemical analysis. Immunofluorescence analyses of mice hippocampi revealed that RTP801 knockdown in neurons tends to decrease the number of Sox2+ cells in the subgranular zone (SGZ) of the DG. In this line, the number of mature NeuN+ neurons increases, independently of the genotype. Altogether results suggest that neuronal silencing of RTP801 increases the differentiation of neural stem cells (NSCs) of the SGZ to mature neurons of the granular cell layer, thereby increasing the number of neurons. This new putative role of RTP801 paves the way for further studies aimed to unravel the significance of such process but already suggests an important role in migration and differentiation of NSCs in AHN.

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P.60. CHIMERIC CHRONOKINE HEBE FOR ALZHEIMER'S AND AGE-ASSOCIATED NEURODEGENERATIVE DISEASES: TESTING DOMAIN ACTIVITIES AND PROMOTER SUITABILITY FOR A GENE THERAPY APPROACH.

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Chronokines have emerged as a promising tool to tackle age-associated pathologies, as their longterm expression has been shown to provide beneficial eMects on such diseases without toxicity or adverse side-effects. In this context, and with a focus on a multifactorial disease such as Alzheimer's (AD), our group has engineered a new chimeric protein named HEBE, comprising the chronokines sTREM2, sKL and TIMP2, with the aim of targeting multiple AD-aMected pathways simultaneously. Due to the chronic nature of this CNS-aMecting disease, a gene therapy approach for HEBE has been designed, which allows for long lasting therapeutic compound production after a single administration timepoint. For this, a specific variant of the AAV9 viral vector will be used, capable of crossing the blood-brain barrier when injected intravenously. This, and because of the limited packaging capacity that AAV vectors oMer, combined with the large size of HEBE, makes it necessary to evaluate diMerent small promoter sequences, such as the CBh, which allow for robust and durable expression levels. In this context and prior to HEBE administration in vivo, the principal objectives of this study were: i) to evaluate whether the activity of each individual domain is maintained in HEBE, and ii) to assess the applicability of the CBh promoter for an HEBE intravenous CNS-targeted gene therapy strategy. The principal findings of this study demonstrate that two out of the three domains of HEBE retain their activity in the chimeric conformation, while tests for the third domain are underway. Furthermore, the CBh promoter supports correct HEBE expression, and when compared to the CMV, achieves higher reporter gene levels in mice brains and higher mRNA expression in liver. Altogether, this proof of concept sets the starting point for the development of an HEBE-based therapy to tackle neurodegenerative diseases.

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P.61. PRODUCTION OF STRESS-REGULATED PROTEIN RTP801 INHIBITORS THROUGH IN-SILICO AND IN-VITRO TESTING.

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RTP801 is a stress-regulated protein involved in the mTOR pathway. RTP801 has been found to be involved in various neurodegenerative diseases, including Huntington's disease (HD), Alzheimer's disease (AD) and Parkinson's disease (PD). Moreover, RTP801 is upregulated in postmortem samples of AD patients and selective knockdown of its expression improves the neuroinflammatory and cognitive profiles in a murine model of AD. The present work focuses on the research of potential RTP801 inhibitors. A molecular simulation study using DruGUI, complemented with the DoGSiteScorer module of ProteinPlus, was used to predict binding pockets of the RTP801 structure. Two different docking software programs were used to perform a consensus docking approach of more than 280.000 compounds with RTP801. Results were further filtered through maximising the electrostatic complementarity value and the chemical diversity between compounds. This led to the selection of 20 compounds as potential RTP801 inhibitors. To test the in vitro activity of the compounds, HEK293T cells were exposed to fixed concentrations of each putative inhibitor. The effects on signaling resulting from such inhibition were quantified by western blotting. Specifically, phospho-S6, phospho-Akt, phospho-4EBP1 and RTP801 were selected as readouts. Moreover, toxicity of the compounds was tested via MTS assay. This work represents the first attempt in finding effective RTP801 inhibitors that could be useful in various neurodegenerative diseases.

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POSTER SESSION - NEUROTRANSMITTER RECEPTORS AND SIGNALING

P.62. GRIN-RELATED DISORDERS GENETIC MOUSE MODELS PHENOTYPIC ASSESSMENT AND PHARMACOTHERAPY TRANSLATION INTO CLINICAL PRACTICE

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One decade ago, next generation sequencing techniques allowed to identify the association between de novo mutations affecting GRIN genes (encoding for the NMDAR subunits) and the so-called GRIN-related disorders (GRD, GRINopathies) developmental encephalopathies. Beyond the use of cellular models to interrogate the functional consequences of GRIN variants, there is an urgent need to develop and to characterise GRD animal models covering the genetic and functional scenarios associated with GRD, to further evaluate the putative therapeutic and tolerability effects of novel GRD treatments. To address this clinical need, we built-up a small in vivo library of genetic mouse models of GRD, consisting of haploinsufficient mouse models Grin2a+/- and Grin2b+/- paradigmatic of GRIN2A and GRIN2B loss-of-function variants respectively, together with the KI-Grin2a(S1048D) mice, a likely predicted GRIN2A gain-of-function (GoF) mouse model. The comprehensive phenotypic assessment was completed for Grin2b+/-, and revealed that Grin2b haploinsufficiency alters hippocampal synaptoproteome, causes synaptic plasticity deficits (LTP disturbance) and motor and cognitive impairment. Similarly, KI-Grin2a(S1048D) GoF mice showed mild hippocampal phenotypes, both in brain slices and in cognitive tasks performance. Importantly, Grin2b+/- endophenotypes were used to investigate the potential therapeutic effect of a positive allosteric modulator (PAM) of GluN2B subunit-containing NMDARs. Chronic PAM administration in young adult Grin2b+/- mice partially rescued hippocampal long-term potentiation deficits in hippocampal slices of Grin2b+/- mice, and improved learning and memory. Based on these preclinical findings, a case study was conducted in two paediatric patients harbouring GRIN2B-LoF variants. Remarkably, one year PAM precursor treatment improved patients' adaptive behaviour, with the absence of noticeable side effects. Overall, these findings show the suitability of genetic mouse models of GRD for preclinical screening, as well as their use for defining novel therapeutic arms for GRD precision medicine.

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P.63. A NOVEL COMPUTATIONAL MODEL FOR MISSENSE VARIANTS PATHOGENICITY PREDICTION

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Recent advances in genome analysis released a bulk of millions of genetic variants, with single nucleotide variants (SNVs) emerging as the most prevalent type of genetic variation among humans. While SNVs are mostly neutral polymorphisms, a subset consists of disease-causing missense variants implicated in autosomal dominant disorders. In the context of patient diagnosis, identifying rare disease-causing missense variants and their functional outcomes is experimentally challenging, and computational methods might be highly convenient to reduce the gap between genetic and functional studies. In this study, we have developed a novel computational approach to predict genetic variants pathogenesis, by studying whether missense variants pathogenicity can be extrapolated to homologous variants, i.e., variants affecting the same position in homologous proteins and exhibiting the identical or similar amino acid change. With this purpose, we extracted homologous variants in a dataset composed of 1,301,690 human missense pathogenic variants from ClinVar and 16,412,219 neutral missense variants from gnomAD. From these, we identified 3,775 pairs of homologous variants in proteins involved in autosomal inheritance diseases, from which 1,433 pairs were both disease-causing, 2,164 pairs were both neutral and 178 pairs disagreed in pathogenicity annotation, achieving an error rate of 4.72%. Further, this new approach was used to predict the pathogenesis of GRIN variants previously identified in patients with a neurological condition. The computational approach was experimentally validated, and duplicated the number of annotated GRIN variants, reduced by 30% GRIN variants with uncertain significance, and increased by 70% of functionally annotated GRIN variants. Our findings strongly support the concept that pathogenicity can be reliably extrapolated across homologous variants with a high degree of accuracy. This innovative approach significantly broadens the scope of variants amenable to precise pathogenicity annotation, offering promising implications for clinical diagnostics and therapeutic interventions.

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P.64. COMPREHENSIVE FUNCTIONAL ANNOTATION OF DE NOVO GRIN VARIANTS AND PRECISION MEDICINE OF GRIN-RELATED NEURODEVELOPMENTAL DISORDERS

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Mutations affecting GRIN genes, which encode for the NMDA receptor subunits, cause GRIN-related disorders (GRD), a group of developmental encephalopathies with a clinical spectrum including intellectual disability, hypotonia, ASD and epilepsy. The functional annotation of GRIN de novo variants is critical i) to understand GRD pathophysiology, ii) to evaluate potential therapeutic strategies and iii) to define personalised therapeutic approaches. Traditionally, GRIN variants have been dichotomically classified into those leading to gain- and loss-of-function (GoF and LoF, respectively). Nevertheless, certain GRIN variants result on complex functional phenotypes (coexistence of GoF and LoF biophysical features for a given GRIN variant). Further, variants affecting the intracellular carboxy-terminal domain (CTD) cannot be functionally evaluated using conventional expression systems and a definitive annotation remains elusive too. To address these issues, we have developed a comprehensive functional analysis pipeline of GRIN variants, expanding the in vitro approaches covering the spectrum of de novo GRIN variants functional outcomes complexity. The expanded pipeline comprises the transient expression of orphan GRIN variants into COS7 cells, allowing to determine the potential alteration of surface trafficking of recombinant mutant NMDARs. Further, mutant receptors are expressed in HEK293T cells, towards biochemical analysis, as well as biophysical assessment using whole-cell patch-clamp technique. Overall, these primary readouts allowed to classify de novo GRIN variants into GoF, LoF and complex variants. Further, complex and CTD GRIN variants were transiently expressed in primary neuronal hippocampal neurons established from embryonic mice. In this model, we have developed several cellular and electrophysiological readouts (spontaneous NMDAR-mediated excitatory postsynaptic currents, chemical LTP) that provided a comprehensive picture of the functional impact of complex and CTD GRIN variants, as well as screening readouts for personalized therapies. In summary, this work shows the new advances into the systematic annotation of GRIN variants and the pharmacological screening of personalised treatments.

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P.65. CHRONIC (S)-KETAMINE IN MICE PROMOTES CHANGES IN OPIOID RECEPTOR EXPRESSION AND FUNCTION

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Ketamine is a chiral compound with two active enantiomers. The discovery of the antidepressant actions of (S)-ketamine led to its approval for treating depressive disorders in patients non-responsive to other pharmacological therapies. However, this approval was not without controversy, given ketamine's potential impact on the reward system and its associated abuse liability. (S)-ketamine is a low potency N-methyl-D-aspartate receptor (NMDAR) non-competitive antagonist, but it also acts as a partial agonist on opioid receptors, such as mu opioid receptor (MOR) and kappa opioid receptor (KOR). Nevertheless, it's mechanism of action is not fully understood, because of this, we aim to investigate S-ketamine's gene expression modulation of NMDAR, MOR, and KOR.
Mice received acute or chronic administration of (S)-ketamine or saline (n=6) and were perfused with ice cold PFA 4%. All brains were collected, post-fixed with 4% PFA for 24h and finally moved to a RNase-free 30% sucrose solution until they completely sunk. Finally, Sections corresponding to the habenula, nucleus accumbens, paraventricular thalamus and VTA were cut at 20 µm using a cryostat and directly mounted on super-frost slides. Tissue from the acute administration cohort was processed for in-situ hybridization (ISH) for MOR, KOR and NMDAR or immunohistochemistry for c-Fos and BDNF. The chronic administration group was processed for ISH for MOR, KOR, and NMDAR. To further understand receptor co-expression function and dynamics, we also used HEK-293 cells expressing MOR and KOR and performed signaling experiments to evaluate the effects of (S)-ketamine on opioid receptor function and trafficking. Chronic S-ketamine altered opioid receptors expression and signaling both in HEK-293 cells and in mice brains. These changes differed across distinct brain areas, were mostly dependent on the subtype of opioid receptor predominantly expressed in the area (MOR or KOR) and the co-expression of the other, indicating an intricate mechanism of action of the drug that could differ under different basal conditions or brain states. Overall, our results suggest that prolonged exposure to S-ketamine can modify gene expression and that MOR and KOR might contribute to S-ketamine's pharmacological effects, as well as the effects of other therapeutic drugs.

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P.66. Expression and function of the C-terminal tail of the dopamine D2 receptor in living cells

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G protein-coupled receptors are comprised of seven transmembrane domains connected by intra- and extracellular loops, with specific functions in ligand binding and signalling transduction. For intracellular loop 3 (IL3), only a small portion of its sequence is required for receptor function, and large deletions of this segment have no effect on receptor activation. This was observed for the muscarinic M2 receptor (M2R), which can be split at the IL3 level into two segments, each acting as an autonomous folding protomer capable of reconstituting a physiologically active receptor when expressed together in transfected cells. A mutant of the receptor carrying a stop codon at position 228 in IL3 was surprisingly observed to retain ligand binding and signalling activity. Since the N-terminal fragment can reconstitute a physiologically active receptor when co-transfected with the C-terminal fragment, a plausible possibility is that the C-terminal segment of the receptor can be independently produced and reconstitute an active receptor with the N-terminal portion on the cell membrane. A recent study demonstrates that the C-terminal domain of M2R is translated by the ribosome through an internal ribosome entry site (IRES). Given the similarity between the dopamine D2 receptor (D2R) and M2R nucleotide sequences at the IRES level, we split D2R into two protomers and studied whether its activity could be reconstituted. A stop codon at position 283 of the IL3 was inserted, and the resulting stopped mutant gave rise to a trunk fragment, while no synthesis of a C-terminal fragment was observed. However, the use of antibodies targeting the C-terminal of D2R gave some clues that C-terminal fragments could be synthesised independently of complete receptor translation, as observed for M2R.

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P.67. ALEX3/GAQ PROTEIN COMPLEX REGULATES MITOCHONDRIAL TRAFFICKING, DENDRITIC COMPLEXITY, AND NEURONAL SURVIVAL

Manso Y [1,2,*], Izquierdo-Villalba I [3*], Mirra S [1,1,2,4,5,6*], Parcerisas A [1,2,7], Rubio J [3], Del Valle J [2,8], Gil-Bea F J[2,9], Ulloa F [1,2], Herrero-Lorenzo M [1], Verdaguer E [1,2], Benincá C [3], Castro-Torres RD [1], Rebollo E [3], Marfany G [4,5,6], Auladell C [1,2], Navarro X [2,8], Enríquez JA [10,11], López de Munain A [2,9,12], Soriano E [1,2,#], Aragay AM [3#].

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Mitochondrial dynamics and trafficking are essential to provide the energy required for neurotransmission and neural activity. We investigated how GPCR and G proteins control mitochondrial dynamics and traffic. The activation of Gαq was found to negatively regulate mitochondrial trafficking in neurons through a mechanism independent of the canonical PLCβ pathway. Mitoproteome analysis revealed that Gαq interacted with the Eutherian-specific mitochondrial protein Alex3 and the motor-adaptor Miro1/TRAK2 complex, which traffics mitochondria along dendrites and axons. By generating a CNS-specific armcx3 knockout mouse line, we demonstrated that Alex3 is required for Gαq effects on mitochondrial trafficking and dendritic growth. Alex-3 deficient mice presented altered ER stress response protein levels, correlating with increased neuronal death, motor neuron and neuromuscular synaptic loss, and severe motor alterations. These data revealed a novel mammalian-specific Alex3/Gαq mitochondrial complex which enables control of mitochondrial trafficking and neuronal death by GPCRs, and could open novel therapeutic venues to target mitochondria in neurodegenerative diseases such as Parkinson or neuromuscular disorders.

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P.68. ALTERED ACTIVITY-DEPENDENT NEURONAL GENES IN ALZHEIMER'S DISEASE

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Changes in gene expression programs in memory-related neural circuits are associated with cognitive decline in normal and pathological aging, including Alzheimer's disease (AD). However, the specific genes and the transcriptional regulatory mechanisms underlying synapse and cognitive dysfunction during AD progression are largely unknown. Our previous studies have revealed impairment of activity-induced CREB/CRTC1-regulated transcription at early and intermediate AD pathological stages. The aim of this study is to examine the mechanisms of activity-dependent gene changes during AD pathological progression.
We identified potential activity-regulated genes by analyzing published RNA-seq and CREB/CRTC1 ChIP-seq datasets obtained from depolarized mouse cultured neurons. We applied biochemical and molecular biology approaches to assess the differential expression of gene transcripts and proteins induced by neuronal activity and potentially altered in human hippocampus of AD. Furthermore, we used lentiviral transduction to modulate CREB signaling in mouse neuronal cultures.
Our results revealed that specific activity-dependent immediate-early-genes (IEGs) as well as synaptic function and plasticity-related genes were transcriptionally regulated by CREB/CRTC1 signaling. Accordingly, pharmacological and lentiviral modulation of the CREB/CRTC1 signaling pathway confirmed differential modulation of these activity-dependent genes in primary neurons. Importantly, some of these genes were deregulated in the human hippocampus at distinct AD pathological stages.
These results indicate that altered expression of activity-dependent neuronal genes regulated by CREB/CRTC1 in memory-related neural circuits may contribute to early cognitive dysfunction in AD.

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P.69. BRAIN BIOLUMINOLYSIS OF A G PROTEIN-COUPLED RECEPTOR PHOTODRUG

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Photopharmacology is an emerging approach that allows the spatiotemporal control of receptor function using photodrugs and offers superior tissue specificity and reduced off-target effects. Previously, we successfully demonstrated the blockade the adenosine A2A receptor (A2AR) in mouse brain using MRS7145, a photocaged derivative of the selective A2AR antagonist SCH442416 (1). Here, we explore the use of bioluminescence resonance energy transfer (BRET) to photouncage MRS7145 in vitro and in vivo. First, we demonstrated the effective release of SCH442416 by BRET uncaging or bioluminolysis of MRS7145 in HEK-293 expressing A2AR tagged with nanoluciferase (NL) at the N-terminus (i.e., A2ARNL). Bioluminolysis-mediated A2AR blockade was monitored by cAMP accumulation determinations after CGS21680 activation. Next, we expressed A2ARNL in the mouse brain using an adeno-associated virus (AAV). Correct expression of A2ARNL in the mouse striatum was confirmed by luminescence recordings and immunohistochemical detection of A2ARNL. The photochemical properties and pharmacokinetics of several NL substrates for in vivo use in behaving mice were characterized. Finally, the bioluminolysis activation of MRS7145 in living animals is tested through several behavioural tests, namely locomotor activity and anticataleptic activity. Our method shows potential to improve animal behaviour studies by eliminating the requirement of restraining cables or implantation of optical fibres.

(1) Taura, J. et al. Remote control of movement disorders using a photoactive adenosine A 2A receptor antagonist. Journal of Controlled Release 283, 135–142 (2018).

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P.70. ELECTROCONVULSIVE SEIZURES MITIGATE PSYCHOTIC-LIKE PHENOTYPE IN MICE LACKING ADENOSINE A2A RECEPTOR

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Schizophrenia presents as a complex psychiatric disorder characterized by a heterogeneous genetic and neurobiological background. Unfortunately, the prevailing mechanistic remains incompletely elucidated. A dopaminergic basis for schizophrenia has been proposed, implicating an upregulation in dopaminergic neurotransmission. This notion is supported by observations of behavioral hypersensitivity to dopamine receptor-activating drugs in individuals with schizophrenia, as well as postmortem analyses revealing elevated density of dopamine D2 receptors (D2R) in the striatum of schizophrenia patients. Moreover, prior literature has validated the A2A receptor knockout mouse (A2AR-/-) as a preclinical animal model of psychosis.1 Electroconvulsive therapy (ECT) has exhibited significant efficacy as a treatment option for individuals with treatment-resistant schizophrenia (TRS). However, despite its effectiveness, the specific neural mechanisms underlying ECT remain poorly understood. This study aims to investigate whether ECT in A2AR-/- mice can ameliorate psychotic symptoms and to address putative molecular and neurochemical alterations associated with dopamine transmission. As anticipated, A2AR-/- mice exhibited a reduction in basal prepulse inhibition (PPI). Distinctively, immunoblotting analyses revealed elevated density in striatal D2R receptors, while photometry determination described an increased frequency of dopamine peaks per minute, both indicative of dysregulated dopamine dynamics. Remarkably, ECT successfully reversed these abnormalities to a level comparable to that of wild-type mice. Collectively, these findings suggest that ECT not only rescues the psychotic-like phenotype in A2AR-/- mice but is also partially able to restore the altered dopamine dynamics observed in these mice.

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P.71. THE OLFACTORY OLFR-78/51E2 RECEPTOR INTERACTS WITH THE ADENOSINE A2A RECEPTOR. IMPACT OF MENTHOL AND 1,8-CINEOLE ON A2A RECEPTOR-MEDIATED SIGNALING

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Heteromer formation is unknown for the olfactory family of G protein-coupled receptors (GPCRs). We here identified, in a heterologous system, heteromers formed by the adenosine A2A receptor (A2AR), which is a target for neuroprotection, and an olfactory receptor. A2AR interacts with the receptor family51, subfamily E, member 2 (OR51E2), the human ortholog of the mouse Olfr-78,whose mRNA is differentially expressed in activated microglia treated with adenosine receptor ligands. Bioluminescence resonance energy transfer (BRET) assays were performed in HEK-293T cells expressing the human version of the receptors, OR51E2 and A2AR, fused, respectively, to Renilla luciferase (RLuc) and the yellow fluorescent protein (YFP). BRET data was consistent with a receptor-receptor interaction whose consequences at the functional level were measured by cAMP level determination in CHO cells. Results showed an olfactory receptor-mediated partial blockade of Gs coupling to the A2AR, i.e., the effect of the A2AR selective agonist on intracellular levels of cAMP was significantly reduced. Two odorants, menthol and 1,8-cineole, which failed to show Golf-mediated OR51E2 activation because they did not increase cytosolic cAMP levels, reduced the BRET readings in cells expressing A2AR-YFP and OR51E2- Rluc, most likely suggesting a conformational change of at least one receptor. These odorants led to an almost complete block of A2AR coupling to Gs.

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P.72. THE GI AND GS PROTEIN-COUPLED μ-OPIOID-GALANIN GAL1 RECEPTOR HETEROTETRAMER

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Recent studies indicate that heteromers of μ-opioid receptors (MORs) and galanin Gal1 receptors (Gal1Rs) constitute a predominant population of the MOR localized in the ventral tegmental area and mediate the dopaminergic effects of opioids. Allosteric mechanisms in the MOR-Gal1R heteromer determine the ability of Gal1R ligands to decrease the affinity and efficacy of opioids and, importantly, a specific decrease in the potency of methadone. This MOR-Gal1R heteromer-dependent pharmacodynamic property of methadone provided a mechanistic explanation for its weaker dopaminergic effects, blunted euphoric properties, and lower addictive liability as compared with morphine and other opioids. Thus, targeting the MOR-Gal1R heteromer provides a logical approach to fighting the opioid epidemic. However, we do not have any knowledge about the quaternary structure of the heteromer, which is key to understanding the functional and pharmacological mechanisms of heteromerization. Studies with other GPCR heteromers suggest that a predominant structure might be a heterotetramer, with each homodimer coupled to its preferred G protein subtype, and this can determine specific interactions through the interacting plasma membrane effector. The present study reports converging evidence, using a peptide-interfering approach combined with biophysical and biochemical techniques, including total internal reflection fluorescence microscopy, for a predominant homodimeric structure of MOR and Gal1R when expressed individually, and for their preference to form functional heterotetramers when co-expressed. Results show that a heteromerization-dependent change in the Gal1R homodimeric interface leads to a switch in G-protein coupling from inhibitory Gi to stimulatory Gs proteins. The MOR-Gal1R heterotetramer, which is thus bound to Gs via the Gal1R homodimer and Gi via the MOR homodimer, provides the framework for a canonical Gs-Gi antagonist interaction at the adenylyl cyclase level. These novel results shed light on the intense

debate about the oligomeric quaternary structure of G protein-coupled receptors, their predilection for heteromer formation, and the resulting functional significance.

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P.73. TYROSINE HYDROXYLASE ACTIVITY IS FOUND IN THE MITOCHONDRIAL FRACTION FROM RAT BRAIN STRIATUM

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Dopamine (DA) is synthesized in the striatum of vertebrates and the dysregulation of its metabolism is a key event in several brain diseases. DA synthesis is controlled by tyrosine hydroxylase (TH), an enzyme typically described as mainly cytosolic although is also found associated to membranes. The modulation of TH activity includes feedback inhibition by DA and phosphorylation at serine 19, 31 and 40.
To explore the intracellular localization of TH, brain striata from naïve Sprague-Dawley rats were extracted, minced and the mitochondrial and cytosolic fractions were separated. Immunodetection with western blot showed that one third of the TH protein was found in the cytosol while two thirds were bound to the mitochondrial fraction, approximately. Determination by HPLC of L-DOPA, the primary product of TH enzyme, showed that TH activity was found mainly in the mitochondrial fraction, while the cytosolic TH protein showed lack of activity. Assessment of TH phosphorylation in Ser31 and in Ser40 by western blot revealed no significant differences between cytosolic and mitochondrial pools. Mass spectrometry (MS) after co-immunoprecipitation of TH was used to find protein partners of TH, to search clues of its putative association to mitochondria. The results showed a relevant number of mitochondrial proteins which bind to TH, such as ATP synthase subunits, mitochondrial transporters or α-synuclein.
In conclusion, we unexpectedly found TH activity in the mitochondrial fraction to a large extent, while the cytosolic TH protein was apparently mostly inactive. Phosphorylation in Ser31 and Ser40 seem not to be a factor controlling TH activation, since both cytosolic and mitochondrial show the same level of relative phosphorylation in those sites. Moreover, MS revealed a significant number of putative mitochondrial partners of TH.

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P.74. DISCOTIC AMPHIPHILE SUPRAMOLECULAR POLYMERS FOR DRUG RELEASE AND CELL ACTIVATION WITH LIGHT

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The limited efficacy shown by drug delivery systems so far prompts to the development of new molecular approaches to release drugs in a controlled and selective manner. Light is a privileged stimulus for delivery because it can be applied in sharp spatiotemporal patterns and is orthogonal to most biological processes. Supramolecular polymers form molecular nanostructures whose robustness, versatility, and responsivity to different stimuli have generated wide interest in materials chemistry. However, their application as drug delivery vehicles has received little attention. We built supramolecular polymers based on discotic amphiphiles that self-assemble in linear nanostructures in water. They can integrate diverse amphiphilic bioligands and release them upon illumination, acutely producing functional effects in physiological conditions. We devised two strategies for drug incorporation into the photoswitchable nanofibers. In the co-assembly strategy, discotic monomers with and without conjugated bioligands were co-assembled in helicoidal supramolecular fibers. In the drug embedding approach, we integrated a potent agonist of muscarinic receptors into the discotic polymer by noncovalent stacking interactions. This ligand can be released on demand with light ex- and in-situ, rapidly activating the target receptor and triggering intracellular responses. These novel discotic supramolecular polymers can be light-driven drug carriers for small, planar, and amphiphilic drugs.

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P.75. PHOTOSWITCHABLE CARBAMAZEPINE ANALOGS FOR NON-INVASIVE NEUROINHIBITION IN VIVO

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A problem of systemic pharmacotherapy is off-target activity, which causes adverse effects. Outstanding examples include neuroinhibitory medications like antiseizure drugs, which are used against epilepsy and neuropathic pain but cause systemic side effects. There is a need for drugs that inhibit nerve signals locally and on-demand without affecting other regions of the body. Photopharmacology aims to address this problem with light-activated drugs and localized illumination in the target organ. Here, we have developed photoswitchable derivatives of the widely prescribed antiseizure drug carbamazepine. For that purpose, we expanded our method of ortho azologization of tricyclic drugs to meta/para and to N-bridged diazocine. Our results validate the concept of ortho cryptoazologs (uniquely exemplified by Carbazopine-1) and bring to light Carbadiazocine (8), which can be photoswitched between 400-590 nm light (using halogen lamps and violet LEDs) and shows good drug-likeness and predicted safety. Both compounds display photoswitchable activity in vitro and in translucent zebrafish larvae. Carbadiazocine (8) also offers in vivo analgesic efficacy (mechanical and thermal stimulus) in a rat model of neuropathic pain and a simple and compelling treatment demonstration with non-invasive illumination.

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SESSION 3A - NEURAL CIRCUITS AND BRAIN PLASTICITY

P.76. INVESTIGATING EMOTIONAL RECOGNITION IN YOUNG AND AGED MALE AND FEMALE MICE.

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Understanding others' emotions is crucial for effective social interaction, allowing individuals to respond appropriately to emotional cues. Rodents, being social animals capable of perceiving and responding to conspecifics' emotional cues, provide a unique opportunity to study the neuronal mechanisms underlying emotional recognition. This project employs an Affective Discrimination Test to identify brain circuits involved in emotional recognition in young and old adult mice. This task evaluates mice's ability to distinguish between two unfamiliar conspecifics based on their emotional states, as one is neutral and the other one is emotionally altered. Mice successfully distinguished between neutral and stressed conspecifics, subjected to acute restraint stress for 15 minutes, or relieved conspecifics, experiencing 23 hours of water deprivation followed by 1 hour of water restoration. To explore the brain regions activated during this task, “Targeted Recombination in Active Populations” (TRAP2) transgenic mice were employed to genetically tag neuronal ensembles activated during emotional recognition. Our findings demonstrate that young (8-12 weeks), but less clear in aged (9 months), mice can discriminate conspecifics based on their altered affective states. Moreover, in a sex- and age-dependent manner, we identified novel brain regions involved in aversive emotional recognition, including the ventral orbitofrontal cortex, habenula, amygdala, and paraventricular nucleus of the thalamus. Notably, chemogenetic approaches targeting the amygdala identified this specific brain region as a key brain location to modulate emotional recognition. After identifying the amygdala as a significant brain region involved in stress emotional recognition, our aim was to elucidate its input and output neuronal circuits. To achieve this, we have used Cre-dependent anterograde and retrograde viruses in TRAP2 mice. In conclusion, our study combines the Affective Discrimination task in mice with advanced genetic and imaging techniques to shed light on the potential brain circuits involved in emotional recognition, highlighting sex and age as important factors.

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P.77. THE ROLE OF NCAM2 IN ADULT NEURONAL PLASTICITY.

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Adult neuronal plasticity, the nervous system's remarkable ability to adapt and reorganize in response to experiences, is one of the most challenging and promising frontiers in neuroscience research. Adult neurogenesis and synaptic plasticity are thought to be the main mechanisms driving this adaptability and are crucial for learning, memory, and cognitive functions throughout life. At the molecular level, cell adhesion molecules (CAMs) have emerged as key elements in the regulation adult neuronal plasticity playing prominent roles in the control of adult neural stem cells (NSCs) behavior and in synapse formation and maintenance. NCAM2 (OCAM/RNCAM) is a neural cell adhesion molecule important for neuronal morphogenesis, dendritic arborization and synaptogenesis. However, its implications in the regulation of hippocampal neuronal plasticity are poorly investigated.
We first analyzed the effects of NCAM2 protein on the regulation of adult neural stem cells (NSCs) during adult neurogenesis. Our results revealed that regulated levels of Ncam2 are necessary for quiescent NSCs activation, division and neuronal differentiation. Increased levels of NCAM2 lead to a partial arrest of the progenitor cells delaying the normal course of the neurogenic events. Moreover, we investigated the role of NCAM2 in dendritic spines dynamics as one of the main mechanisms of synaptic plasticity. We observed that the disruption of Ncam2 leads to a constriction and a reduction in dendritic spines density. Conversely, upregulated levels of the gene may promote contact stabilization by increasing the size of dendritic spines.
The NCAM2 gene has been associated with neurodevelopmental disorders or neurodegenerative diseases such as Autism Spectrum Disorders, Down syndrome, or Alzheimer's disease. Therefore, the evidence provided in this study could enhance our understanding of the neurogenic and synaptic deficits that occur in these pathologies and open new promising avenues for future research.

P.78. COGNITIVE STIMULATION INCREASES EXPRESSION OF SYNAPTIC PLASTICITY MARKERS AND MAY FAVOUR BRAIN CONNECTIVITY IN TGF344-AD RATS.

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Background: Some individuals maintain their cognitive functions despite having developed pathophysiological markers of Alzheimer's disease (AD). This occurrence, known as Cognitive Resilience, grants a better ability to cope with AD's pathological insults. The objective of this study is to assess whether cognitive stimulation affects brain connectivity and the underlying mechanisms of neuroplasticity.
Methods: The experimental design included a longitudinal study in male and female wildtype (WT) rats and the TgF344-AD (TG) AD rat model. Cognitive stimulation was conducted by periodically performing the Delayed Non-Match to Sample task starting from an early (3 months) or late (11 months) age. Resting state (RS) functional and structural connectomics were assessed at 3, 7, 11, 15 and 19 months. RS networks were calculated by independent component analysis using FSL MELODIC and linear mixed models were used to analyse structural and functional connectomics. Synaptic plasticity markers were evaluated by Western Blot analysis at 19 months.
Results: For the early stimulation group, Western Blot analyses revealed enhanced expression of synaptic plasticity markers (PSD95, pGluR1, TrkB and pS6), in trained TG animals, primarily in males. Preliminary analyses for the late-stimulation group also point towards this enhanced expression, despite training occurring after amyloid deposition.
Structural connectomics showed a significant treatment-genotype interaction for the early-stimulation group ($p<0.05$), preserving integration and segregation over time in male trained WT and TG rats vs their untrained counterparts. Intriguingly, functional connectomics analysis indicated a significant treatment effect ($p<0.01$) only in female rats.
Conclusions: Our results suggest that cognitive stimulation enhances biological pathways related to synaptic plasticity, which favours integration and segregation parameters of brain connectomics in trained TG rats compared to untrained ones. This effect may be higher in male than in female animals.

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P.79. UNRAVELING N-GLYCOSYLATION ROLE IN SYNAPTIC TRANSMISSION OF PURKINJE CELLS.

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N-glycosylation is the most abundant glycosylation type in all eukaryotic cells, characterised by the attachment of oligosaccharides on asparagine residues. Despite little is known about the modulatory effects of N-glycosylation on protein function, there are evidences supporting its relevant role in the control on the biophysical properties of membrane proteins. Congenital Disorders of Glycosylation (CDGs) are a group of 135 diseases caused by alterations in the glycosylation pathways. Most of the CDGs have common neurological symptoms, some of each like PMM2-CDG include cerebellar dysfunction and ataxia. These neural-related symptoms and the abundance of glycosylated synaptic proteins (glutamate receptors, CaV2.1, GABA receptors, cerebellin-1), display the relevance of N-glycans in synaptic transmission. The synaptic glycoproteins reported, and the pathophysiological evidence makethe cerebellum and, more specifically, Purkinje cells (PC) the ideal region to study the synaptic function of N-glycosylation. Hence, our objective is to demonstrate that disruption of the glycosylation process alters the PC synaptictransmission and, consequently the cerebellar function. We developed a pharmacological approach to mimic CDGs. On cerebellar organotypic cultures, we apply the inhibitor Kifunensine (Kf) to block the glycosylation pathway and inhibit complex and hybrid N-glycosylation but allowing high-mannose sugar trees. Since inhibition takes place during glycoprotein biosynthesis, the treatment is maintained long enough to make an effect on the glycosylation pattern of proteins. Then, from immunofluorescence images we evaluate the effects of the Kf treatment on the overall structure of the cerebellum, on the integrity of its cell types, and on the number of inhibitory and excitatory synapses, such as granule cells and Purkinje cells synapses (GC-PC). Finally, by means of patch-clamp on PC we intend to characterize the effects of hypoglycosylation on the balance between inhibitory and excitatory synaptic transmission which is usually affected on cerebellar pathologies such as ataxia.

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POSTER SESSION - SENSORY AND MOTOR SYSTEMS

P.80. MUSIC EXPOSURE MODULATES OPIOID-MEDIATED EFFECTS IN A MODEL OF CHRONIC PAIN AND OPIOID DEPENDENCE

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Chronic pain is a pathologic condition that has a significant impact on the quality of life. Opioids are the most potent analgesics and are commonly used, but they can induce severe adverse effects like respiratory depression and opioid dependence. It has been proposed the incorporation of non-pharmacological interventions, such as music listening, to reach an effective and safer management of chronic pain. Here, our aim was to investigate whether music can modulate the effects of opioids when used in the treatment of chronic pain. To this end, we used an animal model of chronic pain and opioid dependence. In brief, mice were unilaterally injected into the hind paw with the inflammatory agent complete Freund's adjuvant and, after nine days, they received repeated morphine administration (10 mg/Kg, twice a day, for five days). After 14 days, we conducted different behavioral tests (Von Frey, naloxone-induced withdrawal, open field, elevated plus maze, splash test, tail suspension test) to evaluate analgesia, abstinence, anxiety-like and depression-like behavior. On the other hand, we assessed the modulatory effects of music on opioid reinforcing effects by using the conditioned place preference paradigm. Finally, we aimed at assessing the ability of music to modulate opioid-mediated molecular alterations in different brain areas (i.e. striatum, amygdala) by conducting western blot and immunohistochemistry. Our data indicated that music exposure modulates the behavioral effects of opioids (i.e. reinforcing properties of morphine), and that these effects are related with restoring a number of molecular changes produced by opioid administration. Overall, our findings support the use of music listening as a non-pharmacological adjuvant treatment to modulate opioid drugs in the management of chronic pain.

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P.81. FUNCTIONAL ELECTRICAL STIMULATION SYSTEM FOR REHABILITATION AFTER SPINAL CORD INJURY

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Pre-clinical and clinical studies have demonstrated the effectiveness of sensorimotor rehabilitation to restore some degree of functional recovery in of patients with spinal cord injury. Due to the wide range of activity dependent plasticity interventions that can be applied to the spinal circuits, new studies are focusing on identifying and optimizing synergistic strategies to increase the effectiveness of the intervention and further improve the patient's sensorimotor outcome.
This study aims to evaluate the functional recovery and plastic changes of spinal cord injured rats that receive alternating bilateral functional electrical stimulation of the extensor muscles of the hind limb but do not perform any exercise, compared to animals enrolled in a daily treadmill training. Our results indicate that both experimental groups showed improvement in sensory and motor function after 2 months of treatment compared to injured but untreated animals, being 2 points higher in rehabilitated animals on the BBB scale. However, when comparing the two groups of animals enrolled in a rehabilitation regimen, no electrophysiological or behavioural differences were observed. We conclude that the use of FES is useful to promote spinal plasticity and motor recovery and could be used to replace or add to specific rehabilitation of conventional tasks.

FLAG-ERA JTC 2017 Project RESCUEGRAPH

P.82. DOWNREGULATION OF HAIR CELL-SPECIFIC GENES IN THE VESTIBULAR SENSORY EPITHELIUM AFTER CHRONIC OTOTOXICITY IN RODENTS

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In response to chronic stress, mammalian vestibular hair cells (HC) may be eliminated by extrusion from the sensory epithelium towards the luminal cavity of the labyrinth. Before HC loss begins, the early damage is characterized by synaptic uncoupling and dismantlement of the calyceal junction, the cell adhesion complex formed between type I HCs and calyx afferents. This early pathology associates with the early loss of vestibular function, and both the initial epithelial and functional alterations are reversible. This study was aimed at identifying key gene expression responses associated with these phenomena. We used three animal models of chronic ototoxicity at similar stages of loss of vestibular reflexes: mice and rats exposed to 3,3'-iminodipropionitrile and rats exposed to streptomycin. Shorter and longer exposure times of the rat IDPN model were also assessed. Vestibular sensory epithelia were collected and processed by RNA-seq, so 5 datasets of differentially expressed genes (DEG) between treated and control groups (n=3/group) were obtained. Comparing the resulting lists, we identified 32 genes downregulated in common in all five treated vs. control comparisons. Among these, 24 have been identified to be specifically expressed by HCs (Bdnf, Bmp2, Ntrk3, Xirp2, Ptprq and others), with no information available for the cell-type specificity of the other 8. By immunohistochemistry and confocal microscopy, we observed decreased expression of the proteins encoded by some of these HC-specific genes (PMCA2/Atp2b2, DNER/Dner, Kv1.8/KCNA10). We conclude that vestibular HCs under chronic ototoxic stress downregulate the expression of genes that characterize their mature functional phenotype. The start of this downregulation response associates with the early loss of vestibular function and the histological evidences of calyceal junction dismantlement. We hypothesize that these functional and histological alterations are caused, at least in part, by the gene expression changes.

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P.83. THE CO-ADMINISTRATION OF HEME OXYGENASE 1 AND MOLECULAR HYDROGEN SUCCESSFULLY REDUCES PACLITAXEL-INDUCED NEUROPATHIC PAIN

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Chemotherapy-provoked peripheral neuropathy and the associated affective disorders are important adverse effects in cancer patients, and its treatment is not completely resolved. A recent study reveals a positive interaction between molecular hydrogen (H2) and a heme oxygenase (HO-1) enzyme inducer, cobalt protoporphyrin IX (CoPP), in the inhibition of neuropathic pain provoked by nerve injury.
Nevertheless, the efficacy of CoPP co-administered with hydrogen-rich water (HRW) on the allodynia and emotional disorders related to paclitaxel (PTX) administration has not yet been assessed. Using male C57BL/6 mice injected with PTX we examined the effects of the co-administration of low doses of CoPP and HRW on the mechanical and thermal allodynia, and the anxiodepressive-like behaviors triggered by PTX. Moreover, the impact of this combined treatment on the oxidative stress and inflammation caused by PTX in the amygdala (AMG) and dorsal root ganglia (DRG) were studied. Our results indicated that the antiallodynic actions of the co-administration of CoPP plus HRW are more rapidly and higher than those given by each of them independently administered. This combination likewise inhibited the anxiodepressive-like behaviors related to PTX, normalized the up-regulation of the inflammasome NLRP3 and 4-hydroxynonenal and stimulated the expression of the antioxidant system Nrf2, HO-1, superoxide dismutase 1 and/or glutathione S-transferase mu 1 in the DRG and/or AMG. Thus, showing a positive interaction among HO-1 and H2 systems in controlling PTX-induced neuropathy by modulating the inflammatory and oxidative responds. This study suggests the co-administration of CoPP plus HRW as an effective treatment for PTX-provoked neuropathy and its linked emotive deficits.

Ministerio de Ciencia, Innovación y Universidades, Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea (Grant: PI21/00592)

P.84. TRESK IN MRGPRD+ NEURONS-MEDIATED COLD SENSITIVITY

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TRESK background potassium channel plays a critical role in modulating the action potential firing and excitability of primary sensory neurons. It is selectively expressed in populations of neurons involved in the perception of touch (low-threshold mechanoreceptors) and pain (nociceptors) and its depletion results in enhanced pain sensitivity. Recent studies indicate that TRESK channel reduces mice's cold and mechanical sensitivity. Nevertheless, it is not clear whether it is involved in the cold sensing mediated by neurons from the dorsal root ganglia (DRG).

Using wild type and TRESK knock-out mice, we have explored the role of the channel in a population of DRG nociceptors expressing the MrgprD receptor (MrgprD+ neurons). In a multidisciplinary study, we have validated that TRESK is expressed in 70% of MrgprD+ neurons and that it modulates their excitability, reducing the number of neurons activated by the MrgprD specific agonist β-alanine in male and female mice, and by cold temperatures in female mice. Moreover, we have also observed that TRESK modulates the cold responsiveness of other populations of DRG nociceptors and TRPM8- expressing primary sensory neurons.

Although we have found that TRESK seems to modulate mice's MrgprD+ neurons-mediated cold sensitivity, knocking out the channel does not influence DRG-mediated mice's cold sensitivity. Moreover, we have found that TRESK inactivation by the calcineurin inhibitor Tacrolimus does not affect DRG- mediated mice's sensitivity to cold temperatures. Nevertheless, activating DRG MrgprD+ neurons with β- alanine seems to increase the cold sensitivity of TRESK KO but not WT mice.

In summary, TRESK modulates the excitability of MrgprD+ nociceptors and their activation by cold temperatures. Moreover, the channel seems to participate in the mice's cold sensitivity mediated by MrgprD+ neurons, which is not sufficient to impact mice's DRG-mediated cold sensitivity in physiological conditions and in a model of calcineurin inhibitor-induced pain syndrome.

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P.85. EXPLORING THE LINK BETWEEN CPT1C DEFICIENCY, AMPA RECEPTORS EXPRESSION AND NOCICEPTION

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In recent years, pain research has experienced an exponential growth. However, despite these advancements, our understanding of this very complex subject remains far from comprehensive. The majority of research efforts in the field of nociception have focused on elucidating the role of ion channels and receptors in sensory and spinal cord (SC) neurons. Conversely, there has been relatively less emphasis on exploring the involvement of intracellular proteins in this phenomenon. One such protein is carnitine palmitoyltransferase 1C (CPT1c), which plays multiple roles in different processes ranging from learning and memory to lipid metabolism. Particularly relevant to our study is CPT1c's ability to modulate the surface expression of AMPA-type glutamate receptors by interacting with them within the endoplasmic reticulum, specifically with the GluA1 subunit. In this study, we explore the potential role of CPT1c in modulating nociception through the regulation of AMPARs in both sensory neurons from the dorsal root ganglia (DRGs) and spinal cord neurons.

Electrophysiological recordings of small-diameter DRG neurons show hyperexcitability with no changes in action potential (AP) threshold. Analyses of APs also indicate an elevated afterhyperpolarization phase in CPT1c-deficient nociceptors. Intracellular calcium mobilization in response to AMPA and glutamate stimulation are enhanced in CPT1c KO SC neurons in culture. Consistent with these findings, AMPA- evoked currents from cultured SC neurons are also slightly increased in the KO group. We found a decrease in GluA1 expression in the SC of CPT1c female animals, but not in males. Additionally, samples from KO males exhibit a significant reduction in GluA2 expression. Lastly, CPT1c-deficient animals exhibit enhanced sensitivity to mechanical stimuli, especially in females.

Taken together, our findings suggest that the increased sensitivity observed in KO animals may result from enhanced nociceptive input from the periphery combined with alterations in AMPAR composition in the spinal cord, ultimately impacting neurotransmission and nociception.

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P.86. TRESK CHANNEL REGULATES SENSORY NEURON EXCITABILITY AND ENHANCES ACUTE AND CHRONIC ITCH

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TRESK (K2P18.1) is a background potassium channel expressed in sensory neurons, where it modulates the resting membrane potential, action potential firing and neuronal excitability. Some of these neurons expressing specific TRPs and Mas-related G protein-coupled receptors (Mrgprs), are activated by pruritogens and mediate itch sensations. Given TRESK's role in somatosensation and pain perception, we investigated it in pruritic sensitivity and its potential for treating chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma and different types of dermatitis. Through RNA in situ hybridization, calcium imaging, electrophysiological and behavioral approaches, we found that TRESK is involved in non-histaminergic itch modulation. In situ hybridization experiments show that TRESK coexpresses with mouse MrgprD+ and MrgprA3+ in sensory neurons. In behavioral experiments, intradermal injection of chloroquine (CQ), a MrgprA3 agonist, produced an acute scratching response in the cheek model, which was significantly enhanced in mice lacking TRESK.
Interestingly, TRESK knockout (KO) mice also showed alterations in different models of chronic itch such as Psoriasis, Allergic Contact Dermatitis or Dry Skin, where TRESK KO mice showed a significantly higher scratching response compared to wild-type (WT).
Behavioral tests of acute itch corroborate that cloxyquin acts as a specific TRESK activator, where intraperitoneal pre-treatment did not produce any effect in TRESK KO mice, whereas WT mice showed significantly reduced scratching responses. Furthermore, in a mouse model of imiquimod-induced psoriatic itch, cloxyquin appeared to decrease spontaneous scratching episodes in WT mice compared to TRESK KO. Additionally, in situ hybridization of human dorsal root ganglia (DRG), also showed coexpression of TRESK and MrgprX1 (mice MrgprA3 homologue).
In summary, our data indicate that TRESK is involved in regulating the excitability of sensory neurons that mediate histaminergic-independent itch. Given the prominent role of this neuronal subpopulation in chronic itch diseases, TRESK emerges as a potential candidate for therapeutic intervention.

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P.87. FUNCTIONAL ANALYSIS OF KCNK18 GENETIC VARIANTS ASSOCIATED WITH NEUROPATHIC PAIN

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Neuropathic pain (NeuP) arises from lesions or diseases affecting the somatosensory nervous system, with origins ranging from trigeminal neuralgia to diabetic neuropathy and small-fiber neuropathy (SFN). The complex pathophysiology requires a multidisciplinary approach to study NeuP effectively. Our recent research identified potentially pathogenic novel gene variants in KCNK18 and several transient receptor potential channels (TRPs) in SFN patients. These findings underscore the role of ion channels in pain perception, particularly TRESK encoded by KCNK18, which regulates neuronal activation. Patients commonly report a wide range of abnormal sensations such as stabbing or burning pain, itching, numbness, hyperalgesia, or allodynia. Since pharmacological treatments often offer limited relief, understanding the genetic basis of NeuP is crucial for advancing its management.
The p.(Met370Cysfs*?) and p.(Ser252Leu) KCNK18 variants have emerged as two of the most relevant and potentially pathogenic mutations in the analyzed patient cohorts. Specifically, the heterozygous thymine deletion at c.1107 (p.(Met370Cysfs*?)) results in a frameshift mutation, leading to an extended out-of-frame C-terminus. By quantifying current density with whole-cell patch clamp, we observed a significant reduction of basal current density in the p.(Met370Cysfs*?) TRESK compared to the wild-type TRESK. It may heighten neuronal susceptibility to stimuli, potentially contributing to increased excitability. These findings are further supported by the reduced expression of the p.(Met370Cysfs*?) TRESK at the plasma membrane, as revealed by our confocal microscopy studies. In addition, the p.(Ser252Leu) KCNK18 exhibits a notable reduction in basal current density, possibly attributed to altered channel phosphorylation, suggesting a potential compromise in neuronal excitability. Notably, we have found larger response to intracellular Ca2+ concentration in the p.(Ser252Leu), indicating an altered calcineurin-dependent modulation. We currently aim to elucidate genotype-phenotype correlations to deepen our understanding of the genetic basis of NeuP and to improve its management, ultimately seeking for enhanced treatment outcomes.

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POSTER SESSION - COGNITION AND MENTAL DISORDERS

P.88. FROM SENSORY PRECONDITIONING TO REALITY TESTING: A NEW BEHAVIORAL PROTOCOL TO STUDY PSYCHOTIC-LIKE STATES IN MICE.

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The adaptation of animals and humans to environmental changes relies on the encoding and storage of past experiences. While direct associative learning with a reinforcer has traditionally dominated cognitive studies, recent attention has shifted towards other forms of learning, deemed more prevalent in daily human decision-making.
Particularly, associations between non-reinforced stimuli represent the most evolutionarily way of enhancing the capabilities for behavioral adaptation. These cognitive processes are conserved in several species ranging from rodents to humans and are called higher-order conditioning.
Higher-order conditioning can explain why individuals often exhibit strong attractions or aversions to different stimuli lacking intrinsic value, suggesting that these stimuli were incidentally associated with directly reinforced cues. This is called mediated learning (ML) and, in rodents, can be studied through sensory preconditioning paradigms. These tasks consist in pairing two low-salience stimuli (e.g. odors, tastes, light, tone) during a preconditioning phase, followed by a conditioning phase where one of the stimuli is paired with a mild footshock. This combination results in a conditioned response (i.e. freezing response) to both the conditioned stimulus (direct learning) and the non-conditioned stimulus (ML). Interestingly, with extended number of associations during the preconditioning phase, mediated learning evolves into what researchers define as "reality testing" (RT) facilitating discrimination between stimuli saliences, which its impairment is considered a core feature in psychotic-like states.
Thus, we set up a light-tone sensory preconditioning task in male and female mice. Additionally, we are implementing a RT protocol using this behavioral paradigm in mice. Remarkably, after additional light-tone pairings, mice not only lose ML, but also present latent inhibition, as the ability to learn the relevance of the conditioned stimulus is reduced.
Hence, we aim to use this behavioural protocol to understand what are the neural underpinnings of psychotic-like states induced by psychotogenic drugs such as the delta-9-tetrahidrocannabinol (THC) treatment in mice.

CaixaResearch - Caixa Health Project (HR23-00793)

P.89. BEHAVIORAL, MOLECULAR AND CELLULAR EFFECTS OF LOW-DOSE CBD ADMINISTRATION IN A CHRONIC STRESS-INDUCED MAJOR DEPRESSION DISORDER MOUSE MODEL.

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Major Depressive Disorder (MDD) is a prevalent, persistent and severe psychiatric condition characterized by persistent feelings of depression, anhedonia, and occasional suicidal thoughts, with stress identified as a major trigger. Previous research from our lab has shown changes in mouse emotional and cognitive behavior, and cellular and molecular alterations in some brain areas such as hippocampus, striatum and prefrontal cortex. Nevertheless, the mechanism underlying depression remain elusive, and so, an effective treatment. To tackle this issue, cannabidiol (CBD) treatment has emerged as a promising novel therapeutic strategy. Therefore, we established a chronic unpredictable mild stress (CUMS) mouse model to assess the potential behavioral positive effects by lower-than-usual CBD (1 mg/kg) chronic treatment in the MDD-like phenotype mice by monitoring mouse performance in the open field (OF) test, novel object recognition (NOR) test, tail suspension (TS) test, forced swimming (FS) test, and Ymaze test.
Our results show that a low CBD dose mitigates stress-induced behavioral despair and anxiety. We have also performed a broad morphologic and biochemical characterization. Such characterization includes a gross anatomy evaluation of different brain regions, an examination of the state and morphology of striatal medium spiny neurons, a structural synaptic plasticity analysis in the medial pre-frontal cortex (mPFC) and in the striatum (STR) and a Mass Spectrometry study in both, mPFC and in the STR, to have a wide view of the molecular profiles associated to the CBD treatment in the context of chronic stress. In summary, these results broaden our understanding of the altered pathways in MDD associated to chronic stress, thereby paving the way for prospective advancements in therapeutic interventions.

Conflict of interest: Schibano Pharma sponsored this research.

P.90. THE EFFECTS OF ACUTE AND CHRONIC (S)-KETAMINE TREATMENT ON ANXIETY AND MOTIVATION.

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The NMDA receptor non-competitive antagonist (S)-Ketamine is mostly used as an anesthetic as well as analgesic, additionally has recently been approved for pharmacotherapy of treatment-resistant depression patients. Simultaneously, it is known to be a psychotropic drug with abuse potential and can elicit undesired side effects, possibly due to the partial agonism with opioid receptors. As of now, (S)-Ketamine's antidepressant mechanism of action and its effects on behavior are not fully understood. We therefore performed several behavioral tests on mice after acute and chronic treatment with a subanesthetic dose of S-ketamine reported to has antidepressant-like effects (10 mg/kg) to unveil its effects on locomotor activity, anxiety-like behaviors and motivation to obtain palatable rewards. The chronic treatment consisted of 5 days of (S)-Ketamine injections while the acute had only one injection. The results of the Open-Field Test (OFT) and elevated plus maze (EPM) show a decrease in time spent in the center and the open arms, respectively, in animals acutely and chronically treated with (S)-Ketamine. On operant responding progressive ratio tasks acute and chronic (S)-Ketamine treatment decreased the animals breakpoint, indicating lower reward value assigned to a food reward. These results indicate that acute (S)-Ketamine possibly leads to an increase in anxiety as well as affecting the reward system to process rewarding stimuli. Additionally, our data shows that these effects can not be explained by a engagement of a single pharmacological target but are likely the result of the pleiotropic pharmacology of ketamine.

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P.91. THE ACTIVATION OF THE A2A ADENOSINE RECEPTOR PREVENTS THC-INDUCED INCREASE IN DOPAMINERGIC ACTIVITY IN THE VENTRAL TEGMENTAL AREA IN LATE ADOLESCENT RATS.

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Cannabis use during adolescence is associated with increased susceptibility to psychotic episodes and developing schizophrenia later in life. The main psychoactive compound of cannabis is the delta9-tetrahydrocannabinol (THC) which can increase the release of dopamine (DA) in mesolimbic areas. This THC effect is mediated by the activation of the presynaptic CB1 cannabinoid receptors (CB1R) in GABAergic neurons that project onto the DAergic neurons of the ventral tegmental area (VTA). Previous findings demonstrate that A2AR and CB1R exhibit a reciprocal antagonistic functional interaction, which may be explained by the existence of A2AR-CB1R heteromers. Consequently, an A2AR agonist can decrease the activity of CB1R. In this context, the hypothesis tested in the present study is that the THC-induced increase in the VTA DAergic activity could be modulated by an A2AR agonist (CGS 21680) and that age could be a crucial factor in these effects. For these reasons, we investigate the role played by A2AR in the effects of THC in early adolescence (EA) and late adolescence/early adulthood (LA/EA).
To evaluate this hypothesis, extracellular recordings of DA neurons were carried out by using in vivo electrophysiology in rats. The average firing rate of each identified DA neuron was recorded in the VTA of EA (4 weeks of age) and LA/EA rats (7 weeks of age) after the administration of increasing doses of THC, increasing doses of CGS 21680, or a subeffective dose of CGS 21680 (0.05 mg/kg) followed by an effective dose of THC (2 mg/kg).
Our results demonstrate that intravenous administration of THC induced a significant increase in the DAergic firing in the VTA in a dose-dependent manner exclusively in LA/EA rats. Moreover, CGS 21680 decreased DAergic activity with a dose above 0,15 mg/kg exclusively in LA/EA rats. Finally, LA/EA rats were pretreated with a sub-effective dose of CGS 21680 (0.05 mg/kg) to test how activation of A2AR might contribute to regulating the THC-induced effects in a specific way. Interestingly, the pretreatment with the A2AR agonist reduced the DAergic hyperactivity induced by THC in LA/EA rats. Overall, we demonstrated that an A2AR agonist can block the THC effect in the DAergic neurons in the VTA. Moreover, we identify LA/EA as an age particularly sensitive to THC effects.

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P.92. ASSESSING THE LINK BETWEEN MIGRAINE AND ITS COMORBIDITY WITH MAJOR DEPRESSIVE DISORDER

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Migraine is a neurological disorder characterized by recurrent, debilitating headaches that are accompanied by other symptoms such as nausea, vomiting, photophobia and phonophobia. Migraine often presents with comorbidities, such as depression, that further increase the burden of the disease. Hence, patients who suffer from migraine are more likely to develop depression, and vice versa, but the mechanisms involved in this interaction are largely unknown.

To better understand the shared mechanisms between migraine and major depressive disorder (MDD), C57BL/6J 8-week female mice were used as a control group, or, to establish (i) a model of MDD following the chronic unpredictable mild stress (CUMS) protocol, (ii) a model of chronic migraine induced with recurrent injections of nitroglycerin and (iii) a combination of the MDD model and the migraine model. Moreover, animals were intraperitoneally administered with anti-migraine (olcegepant) and/or anti-depressive (fluoxetine) drugs. Behaviour tests, including Von Frey test, sucrose preference test, open field test and tail suspension test, were performed to assess the development of migraine-like and depressive-like symptoms.

All the groups developed orofacial mechanical allodynia (migraine-like pain) and despair (depressive symptom) which were reversed both with anti-migraine and anti-depressive treatments. Interestingly, the combination of migraine and MDD model group was the only that presented anxiety (depressive symptom). None of the groups showed anhedonic symptoms.

Migraine and MDD share pathophysiological mechanisms that can be studied using preclinical models. The beneficial effects of anti-depressive and anti-migraine treatments in both models open up a promising avenue to develop new therapeutic strategies for both disorders.

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XIII SIMPOSI DE NEUROBIOLOGIA

Barcelona (Catalunya), May 28th -29th, 2024

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Barcelona, May 29th, 2024

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