Societat Catalana de BIOLOGIA

# XII SIMPOSI DE NEUROBIOLOGIA

## Cap a la Medicina Traslacional

neuro SCB

7 i 8 de Juny de 2022

Institut d'Estudis Catalans, Barcelona

**Programa i resums de les comunicacions**

Amb el patrocini de:

## COMITÈ ORGANITZADOR

**Carles A. Saura** (Coordinador), Institut de Neurociències, Universitat Autònoma de Barcelona

**Analía Bortolozzi**, Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC-IDIBAPS

**Francisco Ciruela**, IDIBELL, Institut de Neurociències, Universitat de Barcelona

**Joaquim Egea**, Institut de Recerca Biomédica de Lleida, Universitat de Lleida

**Silvia Ginés**, Institut de Neurociències, Universitat de Barcelona, IDIBAPS

**Josep Saura**, Institut de Neurociències, Universitat de Barcelona, IDIBAPS

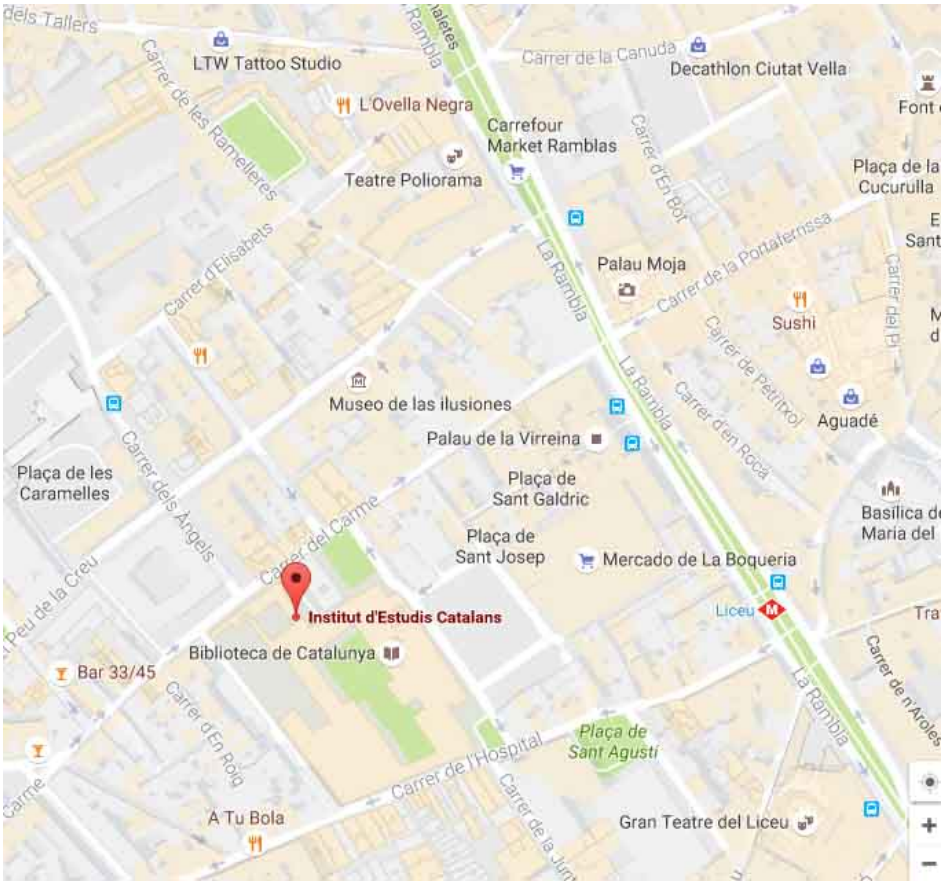
## SECRETARIA TÈCNICA

**Maite Sánchez i Riera**
**Mariàngels Gallego i Ribó**

Societat Catalana de Biologia – Institut d'Estudis Catalans
C. del Carme 47
08001 Barcelona
Tel. +34 933 248 584
e-mail: scb@iec.cat
Web: scb.iec.cat

## LOCALITZACIÓ

**INSTITUT D'ESTUDIS CATALANS**
**Carrer del Carme, 47**
**Barcelona 08001**



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**PROGRAMA**

## Dia 1: 7 Juny de 2022

| | |
|---|---|
| 8.45 h | **Registre i recollida de material** |
| 9.15 h | **Benvinguda i presentació del simposi**<br>Sala: Prat de la Riba |
| 9.30-11.00h | **Sessió 1A** Oral<br>**Cèl.lules glials i inflammació**<br>Sala: Prat de la Riba<br><br>**Sessió 1B** Oral<br>**Desenvolupament del sistema nerviós i malalties relacionades**<br>Sala: Pere i Joan Coromines |
| 11.00-12.30 h | Cafè i sessió de **Pòsters** |
| 12.30-13.30 h | **Conferència plenària 1**<br>**Michael R. Kreutz**<br>**Leibniz-Institute for Neurobiology, Germany**<br>«Protein transport from NMDA receptors to the nucleus in healh and disease»<br><br>Sala: Prat de la Riba |
| 13.30-15.00h | **Dinar i sessió de pòsters** |
| 15.00-16.30 h | **Sessió 2A** Oral<br>**Malalties neurodegeneratives**<br>Sala: Prat de la Riba<br><br>**Sessió 2B** Oral<br>**Receptors de neurotransmissors i neurofarmacologia**<br>Sala: Pere i Joan Coromines |
| 16.30-17.30 h | **Conferència plenària 2**<br>**Josep Dalmau**<br>**IDIBAPS, Hospital Clínic de Barcelona**<br><br>«Autoimmune synaptic diseases»<br>Sala: Prat de la Riba |

## Dia 2: 8 Juny de 2022

| | |
|---|---|
| 9.00 h | **Registre** |
| 9.30-11.00 h | **Sessió 3A** Oral<br>**Circuits neuronals i plasticitat cerebral**<br>Sala: Prat de la Riba<br><br>**Sessió 3B** Oral<br>**Biomarcadors, mecanismes i teràpies**<br>Sala: Pere i Joan Coromines |
| 11.00-12.30 h | Cafè i sessió de **Pòsters** |
| 12.30-13.30 h | **Conferència plenària 3**<br>**Víctor Borrell**<br>**Instituto de Neurociencias de Alicante**<br>«Neurological diseases and the evolution of cortical development»<br><br>Sala: Prat de la Riba |
| 13.30-15.00 h | **Dinar** i sessió de **pòsters** |
| 15.00-16.45 h | **Sessió 4A** Oral<br>**Desordres mentals i comportament**<br>Sala: Prat de la Riba<br><br>**Sessió 4B** Oral<br>**Sistemes sensorial i motor**<br><br>Sala: Prat de la Riba |
| 16.45-17.45 h | **Conferència plenària 4**<br>**5th Ramón Turró Award Conference**<br>**Prof. Guadalupe Mengod**<br>**CIBERNED, IIBB, CSIC-IDIBAPS**<br>«Una història personal dels receptors de serotonina a Barcelona»<br>Sala: Prat de la Riba |
| 17.45-18.00 h | **Premis Millors Pòsters**<br>**Clausura** |
| 18.00 | **FESTA DE CLAUSURA** |

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**PROGRAMA**

## Day 1. June 7th, 2022

| | |
|---|---|
| 8.45 h | **Registration** |
| 9.15 h | **Welcome presentation**<br>Room: Prat de la Riba |
| 9.30-11.00 h | **Session 1A** Oral<br>**Glial cells and inflammation**<br>Room: Prat de la Riba<br><br>**Session 1B** Oral<br>**Neurodevelopment and related diseases**<br>Room: Pere i Joan Coromines |
| 11.00-12.30 h | Coffee and **Poster session** |
| 12.30-13.30 h | **Plenary lecture 1**<br>**Michael R. Kreutz**<br>**Leibniz-Institute for Neurobiology, Germany**<br>«Protein transport from NMDA receptors to the nucleus in healh and disease »<br>Room: Prat de la Riba |
| 13.30-15.00 h | **Lunch and poster session** |
| 15.00-16.30 | **Session 2A** Oral<br>**Neurodegenerative diseases**<br>Room: Prat de la Riba<br><br>**Session 2B** Oral<br>**Neurotransmitter receptors and neuropharmacology**<br>Room: Pere i Joan Coromines |
| 16.30-17.30 h | **Plenary lecture 2**<br><br>**Josep Dalmau**<br>**IDIBAPS, Hospital Clínic de Barcelona**<br>«Autoimmune synaptic diseases»<br>Room: Prat de la Riba |

## Day 2. June 8th, 2022

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|---|---|
| 9.00 h | **Registration** |
| 9.30-11.00 h | **Session 3A** Oral<br>**Neural circuits and brain plasticity**<br>Room: Prat de la Riba<br><br>**Session 3B** Oral<br>**Disease biomarkers, mechanisms and therapies**<br>Room: Pere i Joan Coromines |
| 11.00-12.30 h | Coffee and **Poster session** |
| 12.30-13.30 h | **Plenary lecture 3**<br>**Víctor Borrell**<br>**Instituto de Neurociencias, Alicante**<br><br>«Neurological diseases and the evolution of cortical development»<br><br>Room: Prat de la Riba |
| 13.30-15.00 h | **Lunch and poster session** |
| 15.00-16.45 h | **Session 4A** Oral<br>**Mental and behavioral disorders**<br>Room: Prat de la Riba<br><br>**Session 4B** Oral<br>**Sensory and motor systems**<br><br>Room: Pere i Joan Coromines |
| 16.45-17.45 h | **Plenary lecture 4**<br>**5th Ramón Turró Award Conference**<br>**Prof. Guadalupe Mengod**<br>**CIBERNED, IIBB, CSIC-IDIBAPS**<br>«A personal story of serotonin receptors in Barcelona»<br>Room: Prat de la Riba |
| 17.45-18.00 h | **Best Posters Awards**<br>**Closing remarks** |
| 18.00 h | **CLOSING PARTY** |

## DAY 1: Tuesday, June 7th 2022

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|---|---|
| 8.45-9.15 | **REGISTRATION** |
| 9.15 | **WELCOME PRESENTATION**<br>*Prat de la Riba Room*<br>Carles A. Saura |
| 9.30-11.00 | **ORAL SESSIONS**<br>**Session 1A. GLIAL CELLS and INFLAMMATION**<br>*Prat de la Riba room*<br><br>**Chair: Coral Sanfeliu (Institut d'Investigacions Biomèdiques de Barcelona, CSIC)**<br><br>O.1. Targeting microglia-neuron crosstalk in neurodegeneration. **Puigdellívol M**<br>O.2. Characterization of microglia behavior (morphology and phagocytosis) in healthy and pathological conditions with image analysis tools. **Petegnief V**<br>O.3. Dissecting the role of IFN type I signaling on microglial response in mitochondrial disease. **González-Torres, M**<br>O.4. Lafora disease: neuroinflammation and autophagy in glycogen-induced neurodegeneration. **Durán J**<br>O.5. Green tea and cocoa: Beneficial effects on age-associated regressive changes in the mouse neuromuscular System. **Gras S**<br>O.6. Discovery of genes involved in the microglia-OPCs crosstalk during demyelination and remyelination. **Enrich-Bengoa J**<br><br>**Session 1B. DEVELOPMENT AND RELATED DISEASES**<br>*Pere i Joan Coromines Room*<br><br>**Chair: Neus Pedraza (IRB Lleida, Universitat de Lleida)**<br>O.7. Specific contribution of Reelin expressed by Cajal-Retzius cells or GABAergic interneurons to cortical lamination. **Manso Y**<br>O.8. The role of the RNA-binding protein Staufen 2 during neurogenesis. **Fernández Moya SM**<br>O.9. Artificial extracellular matrix scaffolds of mobile molecules enhance maturation of human stem cell-derived neurons. **Ortega JA**<br>O.10. Indirect pathway linage-specific alterations from early development in Huntington's disease zq175 embryos. **Vila C** |

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| | O.11. Disrupted in schizophrenia 1 gene (DISC1) as a potential mediator of dermatoglyphic neurodevelopmental markers in schizophrenia. **Sotero A**<br>O.12. Unravelling the endophenotypic characteristics of generated GRIN-related disorders Zebrafish models. **Locubiche S** |
| 11.00-12.30 | ☕ **COFFEE BREAK and POSTERS** |
| 12.30-13.30 | **PLENARY LECTURE**<br>*Prat de la Riba Room*<br>Presented by: C. Saura<br>**«Protein transport from NMDA receptors to the nucleus in healh and disease»**<br><br>Michael R. Kreutz (Leibniz-Institute for Neurobiology, Magdeburg. Center Molecular Neurobiology, Hamburg, Germany) |
| 13.30-15.00 | 🍽 **LUNCH** |
| 15.00-16.30 | **ORAL SESSIONS**<br><br>**Session 2A. NEURODEGENERATIVE DISEASES**<br>*Prat de la Riba room*<br><br>**Chair: Eulàlia Martí (Institut de Neurociències, Universitat de Barcelona, IDIBAPS)**<br><br>O.13. Single-cell transcriptomics of iPSC-derived neurons reveals functional changes in Alzheimer's disease. **Gutierrez A**<br>O.14. Differential effects of amyloid-β and tau neuropathology on cognitive and emotional symptoms in novel Alzheimer's disease transgenic mica. **Capilla-López MD**<br>O.15. Is RTP801/REDD1 involved in tRNA processing ? **Campoy G**<br>O.16. An extracellular small RNA biosignature in plasma identifies premanifest Huntington´s disease. **Herrero Lorenzo M**<br>O.17. In vivo reduction of age-dependent neuromelanin accumulation mitigates features of Parkinson's disease. **Compte J**<br>O.18. The Y172-related protein: a new actor in motoneuron physiopathology**. Gatius A** |

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| | **Session 2B. NEUROTRANSMITTER RECEPTORS and NEUROPHARMACOLOGY**<br>*Pere i Joan Coromines Room*<br><br>**Chair: Alex Bayés (Institut de Recerca, Hospital Santa Creu i Sant Pau)**<br>O.19. Position matters: a differential modulation of AMPARs depending on the auxiliary subunit location into the receptor complex. **Soto D**<br>O.20. Comprehensive delineation and precision medicine of GRIN-related neurodevelopmental disorders, a primary disturbance of the NMDA receptor. **Altafaj X**<br>O.21. Remote local photoactivation of morphine produces analgesia without opioid-related adverse effects. **López-Cano M**<br>O.22. Experimental and computational analysis of biased agonism on full-length and a C-terminally truncated adenosine A2A receptor. **Reyes-Resina I**<br>0.23. Screening of adenosine A1 receptor ligands as heteromer-selective ligands for the adenosine A1-dopamine D1 receptor oligomer. **Llopart N**<br>O.24. Nutrient-mediated regulation of GLUA1 surface levels. **Rojas R** |
| 16.30-17.30 | **PLENARY LECTURE**<br>*Prat de la Riba Room*<br>Presented by: S. Ginés<br><br>**«Autoimmune synaptic diseases»**<br><br>Josep Dalmau (IDIBAPS, Hospital Clínic de Barcelona) |

## DAY 2: Wednesday, June 8th 2022

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|---|---|
| 9.30-11.00 | **ORAL SESSIONS**<br><br>**Session 3A. NEURAL CIRCUITS and BRAIN PLASTICITY**<br>*Prat de la Riba Room*<br><br>**Chair: Juan Nacher (Universitat de València)**<br><br>O.25. Young neurons for old cortex. **Nacher J**<br>O.26. A Systems biology approach to decipher the synaptic molecular alterations underpinning the intellectual disability caused by SYNGAP1. **Bayés A**<br>O.27. Role of neuron-derived extracellular vesicles in synaptic plasticity. **Solana-Balaguer J**<br>O.28. CRTC1 regulates neuronal activity-dependent gene programs mediating synaptic plasticity and neuronal excitability. **Parra-Damas A**<br>O.29. Hypothalamic pregnenolone mediates recognition memory in the context of metabolic disorders. **Ramírez S**<br>O.30. Neurophysiological characterization of a mouse model of CPT1C deficiency: from synapses to behavior. **Iborra-Lázaro G**<br><br>**Session 3B. DISEASE BIOMARKERS, MECHANISMS AND THERAPIES**<br>*Pere i Joan Coromines Room*<br>**Chair: Izquierdo-Serra M (Universitat de Barcelona)**<br><br>O.31. Novel Cav2.1-modulators to treat hemiplegic migraine. **Izquierdo-Serra M**<br>0.32. Glycogen synthase kinase-3 inhibition affects dopamine metabolism by decreasing tyrosine hydroxylase activity. **Hamdon S**<br>O.33. GPR37 N-terminal domain processing defines autaptic receptor signalling. **Argerich J**<br>O.34. Role of SAC1 in neuronal mitochondrial fission regulation. **Molins A**<br>O35. Treatment of mitochondrial disorders: metabolic intervention for the treatment of complex I deficiencies based on their molecular profiles. **García-Adán B**<br>0.36. Human brain aging induces changes region-specific in lipid profile. **Mota-Martorell N** |
| 11.00-12.30 | **COFFEE BREAK and POSTERS** |

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| 12.30-13.30 | **PLENARY LECTURE**<br>*Prat de la Riba Room*<br>Presented by J. Egea<br>**«Neurological diseases and the evolution of cortical development»**<br><br>Víctor Borrell (Instituto de Neurociencias de Alicante) |
| 13.30-15.00 | 🍽 **LUNCH and POSTERS** |
| 15.00-<br>16.45 | **ORAL SESSION**<br><br>**Session 4A. MENTAL AND BEHAVIORAL DISORDERS**<br>*Prat de la Riba Room*<br><br>**Chair: Raül Andero (Institut de Neurociències, Universitat Autònoma de Barcelona)**<br><br>0.37.PACAP-PAC1R modulates fear extinction via the ventromedial hypothalamus. **Andero R**<br>O.38. Estrogens modulate food addiction signatures in females. **Casadó-Anguera V**<br>O.39. Progressive behavioural and proteomic changes associated with stress and potential non-invasive therapeutic strategies. **Sancho A**<br>O.40. Validation of APP/PS1 mice as a preclinical model of psychosis in Alzheimer's disease. **Gómez-Acero L**<br>0.41. miR-135 as a new target for antidepressant therapy: preclinical study. **Pavia R**<br>0.42.A zebrafish model of neurotoxicity by binge-like methamphetamine exposure. **Bedrossiantz J**<br>O.43. Disentangling the impact of inhibitory hippocampal neurons in engram formation in Down syndrome. **Sabariego M**<br><br>**Session 4B. SENSORY AND MOTOR SYSTEMS**<br>*Pere i Joan Coromines Room*<br><br>**Chair: Xavier Gasull (Institut de Neurociències, Universitat de Barcelona)**<br><br>0.44. Loss of TRESK background potassium channel enhances acute and chronic itch. **Callejo G**<br>0.45 The interaction between carbon monoxide and hydrogen sulfide during chronic osteoarthritis pain in mica. **Batallé G**<br>O.46. Cpt1a silencing in AgRP neurons improves physical performance via skeletal muscle remodeling. **Serra D** |

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| | O.47. Cannabidiol ameliorates mitochondrial disease via the activation of PPARγ. **Puighermanal E**<br>0.48. Mitochondrial dysfunction in Rett Syndrome: studying a neurological disorder from synaptic metabolism perspective to find new treatment options. **Musokhranova U**<br>O.49. PKA-dependent SNAP-25 and Syn-1 phosphorylation are differently regulated by the neuromuscular activity. **Polishchuk A**<br>O.50. Characterization of the autophagy flux in complex neuropediatric movement disorders to define new personalized therapies. **Serradell A** |
| 16.45-17.45 | **5th RAMON TURRÓ AWARD** honoring the most cited article in Neurobiology performed in Catalunya published in 1994-95: ¨Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors¨. Mol Brain Res 23, 163-178 (1994)<br><br>*Prat de la Riba Room*<br><br>Presented by J. Saura<br>**ACCEPTANCE LECTURE**<br><br>**«A personal story of serotonin receptors in Barcelona»**<br><br>Prof. Guadalupe Mengod (CIBERNED, IIBB, CSIC-IDIBAPS, Barcelona) |
| 17.45-18.00 | **BEST POSTERS AWARDS** to the 3 best posters presented at the XII Symposium<br><br>*Prat de la Riba room*<br><br>**CLOSING SPEECH**<br><br>Carlos A. Saura |
| 18.00-20.00 | **CLOSING PARTY** |

## INDEX

# RESUMS

# DE LES COMUNICACIONS ORALS

# I PÒSTERS

## PLENARY LECTURES

### L.1. PROTEIN TRANSPORT FROM NMDA RECEPTORS TO THE NUCLEUS IN HEALTH AND DISEASE

**KREUTZ MR**

Leibniz Group 'Dendritic organelles and synaptic function', ZMNH, Hamburg, Germany
*Center Molecular Neurobiology Hamburg/ Magdeburg, Germany*

In the past decade we have proposed mechanisms of activity-dependent transport of synaptic proteins to the nucleus. NMDA-receptors (NMDAR) play a crucial role in plasticity-related gene expression and NPlast has shown that the NMDAR complex, which consists of about 70 different proteins, is a rich source of synapto-nuclear protein messenger. In total five of these proteins (Jacob, Abi-1, RNF10, Prr7 and Rack1) were identified by us and our collaborators in various screens and the work done on these proteins has led to the concept that different NMDAR signals induce the nuclear translocation of different proteins. This type of signaling is conceptually appealing because it allows for local encoding of signals at the site of origin and decoding in the nucleus. Yet many questions, factual and conceptual remain: How do synapses differentiate between ongoing synaptic activation and specific activity patterns that drive synapse to nucleus communication? What is the nature of such communication molecules? How do the presumably minute quantities of signaling molecules released from a small number of remote synapses overcome the vast distances from dendrites the soma? How do they retain their integrity and specific properties along the way? In my presentation I will discuss several of these questions.

**NOTES**

## L.2. AUTOIMMUNE SYNAPTIC DISEASES

**DALMAU J**

*ICREA, IDIBAPS, Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona. University of Pennsylvania*

Investigations over the last 15 years have revealed that many neurologic and psychiatric disorders are due to antibody-mediated mechanisms against neuronal proteins and neurotransmitter receptors. The discovery of these remarkable disorders has changed the landscape of how physicians approach the diagnosis and treatment of these patients. Indeed, cases of rapidly progressive memory loss, psychosis, seizures, abnormal movements or impaired level of consciousness previously considered idiopathic are now known to be mediated by antibodies and curable with immunotherapy. In my presentation I will show how the most frequent of these diseases, anti-NMDA receptor encephalitis, was discovered and what we have learned since, including the main clinical manifestations and triggers of the disease. On a more basic level, I will describe the underlying pathogenic mechanisms and show how an antibody can lead to memory deficits or psychosis through a reduction of the levels of NMDA receptors in neurons resulting in changes in synaptic transmission and plasticity. Experience gained from these investigations has led us to discover 11 additional diseases mediated by antibodies against other brain receptors or proteins, each with specific patterns of symptoms and distinct mechanisms. Overall, these studies have resulted in novel treatment strategies that have improved patient outcomes, and are helping us to understand other diseases in which the same receptors are affected by other mechanisms.

**NOTES**

## L.3. NEUROLOGICAL DISEASES AND THE EVOLUTION OF CORTICAL DEVELOPMENT

**BORRELL V**

*Instituto de Neurociencias de Alicante, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Miguel Hernández, St Joan d´Alacant*

The human brain is the result of millions of years of evolution, when genetic mechanisms driving the amplification of neural stem cells during embryonic development were selected, giving rise to a dramatic expansion in size and folding of the cerebral cortex. Intriguingly, cortical expansion and folding were secondarily reversed in some mammalian lineages like rodents, leading to smaller brains with a smooth cortex. The genetic bases of these complex evolutionary dynamics remain unclear. Cortical expansion and folding are key to our cognitive abilities and frequent targets in neurological disease. Our research team studies the cellular and genetic emergence of these mechanisms during evolution as an entry site to understand disease of the developing human brain. While brain expansion in the recent human lineage is in part explained by the emergence of new genes, mounting evidence points at the differential regulation of conserved genetic mechanisms as being central in the evolution of neurogenesis and cerebral cortex size. I will discuss our recent discoveries showing the central role of microRNAs in the regulation of gene expression in the early embryonic cerebral cortex, its critical impact on progenitor cell amplification and neurogenesis, and the consequences of miRNA deregulation in pediatric brain cancer. I will further present our findings on the small non-coding RNA MIR3607, which plays a central role in promoting the amplification of neural stem cells in primates and carnivores via beta-Catenin signaling. Importantly, MIR3607 was secondarily lost in the recent rodent lineage, leading to smaller progenitor cell pools and reduced cerebral cortex size. Our findings demonstrate the central importance of mechanisms regulating gene expression in the evolution of embryonic cortical development, and its consequences on cortex size and in oncogenic brain disease.

*Supported by grants: ERC StG 309633, AEI PGC2018-102172-B-I00, AEI SEV-2017-0723, Fundación AECC*

**NOTES**

## L.4. UNA HISTÒRIA PERSONAL DELS RECEPTORS DE SEROTONINA A BARCELONA

**MENGOD G**

*Institut d'Investigacions Biomèdiques de Barcelona, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona*

This work “Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Mol Brain Res 23, 163-178 (1994) by Pompeiano, M., Palacios, J.M., Mengod, G.” refers to the visualization of the cells in the rat brain that express the mRNA coding for 5HT2A and 5HT2C receptors. It is part of a series of papers published by our two groups, initially at Sandoz AG in Basel Switzerland and later here in Barcelona at the CID of CSIC. It was natural that this begun at Sandoz, in laboratories close to the place were Albert Hofmann synthesized and personally tested LSD in 1938. In the late 1970’s the interest in serotonergic drugs was reflected in the discovery of several subtypes of 5HT receptors using radioligand binding techniques, that in the middle of the 1980’s was expanded by the cloning of up to 14 5HT receptors genes. Our interest was the study of the distribution of these receptors in the mammalian brain that was carried out using two basic techniques: receptor autoradiography and in situ hybridization. This was made possible first by the cDNAs cloning for the 5HT2C (at the time 1C) by Julius et al 1988 followed by that of 5HT2A by Pritchett et al 1988. Our results illustrated the separate nature of these two receptors, emphasized by their different brain distribution.

Which was the interest on these two receptors at that time? The search for new treatments for schizophrenia has been an important challenge since the discovery of Chlorpromazine. The serotonergic component of neuroleptic action was used in the first classification into 5HT1/5HT2 receptors, and later assigned in the “atypical neuroleptics” to 5HT2 receptors. 5HT2 receptors are still the target of intense investigation in the search of new psychedelics for the treatment of depression and other mental disorders.

**NOTES**

## SESSION 1A

## GLIAL CELLS AND INFLAMMATION

### O.1. TARGETING MICROGLIA-NEURON CROSSTALK IN NEURODEGENERATION

**Puigdellívol M[1,2]**

[1] Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona
[2] Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra

There is growing evidence that altered microglia-neuron signaling contributes to synapse and neuronal dysfunction and loss in neurodegeneration which has led to the idea that blocking microglia might be protective in neurodegenerative diseases. However, the underlying mechanisms of microglia-mediated neurodegeneration are unclear and it has been quite established in the field that general blockade of microglia is not a good therapeutic approach. Thus, understanding how to block the detrimental effects of microglia while preserving their beneficial roles is crucial to developing new therapeutic strategies aimed at targeting microglia-neuron crosstalk in neurodegeneration. Microglia-neuron crosstalk involves a plethora of functions that are potential therapeutic avenues in neurodegeneration. These include the engulfment and degradation (phagocytosis) of synapses and neurons by microglia or the modulation of neuronal activity by microglia through the so-called somatic junctions. Microglial recruitment at synapses and neurons as well as phagocytosis can be triggered by “find-me” and “eat-me” signals, such as extracellular nucleotides released from active, apoptotic, or stressed synapses and neurons which in turn activate ADP and UDP receptors on the surface of microglia. Thus, diffusible ADP generates a chemotactic gradient detected by ADP receptors and local UDP triggers phagocytosis via P2Y6R activation. We found that microglia can cause neuronal loss by phagocytosis of stressed-but-viable neurons in some pathological conditions and demonstrated that blocking microglial phagocytosis by inhibition or knock-out of the phagocytic receptor P2Y6 prevented neuronal loss and memory deficits in models of neurodegeneration. Interestingly, microglial processes contacting neuronal bodies at the so-called somatic-neuron junctions have been proposed to regulate neuronal activity via ADP receptors. We found that blocking ADP receptors on microglia ameliorates spatial memory deficits and motor dysfunction in a novel mouse model of Alzheimer’s disease, the APP/Tau mice. Altogether, these results suggest that blocking microglial UDP and ADP receptors may be beneficial in the treatment of neurodegenerative diseases.

*This study was supported by Alzheimer’s Research UK; Innovative Medicines Initiative*

**NOTES**

## O.2. CHARACTERIZATION OF MICROGLIA BEHAVIOR (MORPHOLOGY AND PHAGOCYTOSIS) IN HEALTHY AND PATHOLOGICAL CONDITIONS WITH IMAGE ANALYSIS TOOLS

**Petegnief V**[1,2], Martinez A[3], Hériché J-K[4], Calvo M[5], Tischer C[6], Otxoa de Amezaga A[7] , Bosch A[5], Planas AM[1,2]

[1] Department of Neuroscience and Experimental Therapeutics, Institute for Biomedical Research of Barcelona (IIBB). Spanish Research Council (CSIC).
[2] Institut d'Investigacions Biomèdiques Augustí Pi i Sunyer, Barcelona.
[3] Institute for Bioengineering of Catalonia. Barcelona.
[4] Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany.
[5] Advanced Optical Microscopy Facility, Scientific and Technological Centers, School of Medicine. University of Barcelona.
[6] Centre for BioImage Analysis, European Molecular Biology Laboratory, Heidelberg, Germany.
[7] Achucarro Basque Center for Neuroscience and Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa

Microglia are very sensitive to changes in the environment and respond through morphological transformation, phagocytosis and metabolism adaptations. In order to depict microglia behavior in healthy and pathological conditions, we developed image analysis programs to quantify neuronal death, microglia morphologies and phagocytosis. Primary mice neuron-glia cultures, in which microglia express the tdTomato protein, were exposed to excitotoxic or excitotoxic+inflammatory challenges and analyzed 8h later in time-lapse acquired in a confocal microscope. Neuronal death was assessed by SYTOX staining of nucleic debris and phagocytosis through the engulfment of green SYTOX positive particles in red microglia. We identified 7 morphologies (round, hypertrophic, fried-egg, bipolar and 3 "inflamed" morphologies) and found the morphometric features able to describe them. Through machine learning, we generated a classifier able to separate them and assign one of the 7 classes to microglia in sample images. In control cultures, round and hypertrophic morphologies were the most abundant and excitotoxicity did not promote changes in the distribution of the populations. In contrast, excitotoxicity+inflammation decreased the round and hypertrophic populations and increased the proportion of inflamed morphologies. Excitotoxicity and excitotoxicity+inflammation significantly increased the percentage of phagocytosing microglia to a similar extent. Our data suggest that in vitro accumulation of dead cells is not sufficient at least in our model to significantly modify microglia behavior at early time-points (up to 12h) and that inflammation is critical to promote phenotypical changes in microglia. The tools we generated can be useful to correlate microglia behavior with environmental changes and characterize the phenotype of disease-associated microglia.

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**NOTES**

## O.3. DISSECTING THE ROLE OF IFN TYPE I SIGNALING ON MICROGLIAL RESPONSE IN MITOCHONDRIAL DISEASE

**Gonzalez-Torres M**[1,2], Bianchi P[1], Prada-Dacasa[1], Sanz E[1,2], Quintana A[1,2]

[1] Institute of Neurosciences. Autonomous University of Barcelona, Barcelona, Spain
[2] Department of Cell Biology, Physiology and Immunology. Autonomous University of Barcelona, Barcelona, Spain

Mutations affecting mitochondrial function lead to a group of usually fatal neurodegenerative diseases known as Mitochondrial disease (MD). The pathological mechanisms underlying MD are still not fully understood, leading to a lack of effective treatments. Recently, it has been shown that mitochondrial dysfunction can promote the release of mitochondrial nucleic acids in the cytoplasm, inducing the activation of an aberrant antiviral response. We have recently identified the presence of an interferon type-I (IFN-I)-mediated antiviral-response elicited by mitochondrial double-stranded RNA (mtdsRNA) in mice lacking a subunit of mitochondrial complex I (Ndufs4KO mouse), a well-validated animal model of MD. However, the contribution of this aberrant antiviral response to the pathology, and the cellular players involved, remains unknown. In this regard, microglial cells are major IFN-I responders in the central nervous system and microgliosis is a hallmark of both Ndufs4KO mice pathology and MD, correlating with the disease severity. Thus, to assess the contribution of the IFN-mediated response to microgliosis we have generated a Ndufs4KO mouse lacking IFN-I receptor (Ndufs4:Ifnar1dKO), and isolated microglial cell populations by magnetic-activated cell sorting to achieve transcriptional profiling of these cells. Overall, we aim to provide novel mechanistic insight on the role of IFN-I and microglial responses to mitochondrial dysfunction, paving the way for novel effective treatments for MD.

*Supported by La Caixa Foundation, Ministerio de Universidades, Ministerio de Ciencia e Innovación, European Research Council*

**NOTES**

## O.4. LAFORA DISEASE: NEUROINFLAMMATION AND AUTOPHAGY IN GLYCOGEN-INDUCED NEURODEGENERATION

Pellegrini P[1], Hervera H[2,3,4,5], Varea O[1], del Río JA[2,3,4,5], Guinovart JJ[1,6,7], **Duran J**[1,2,6,8]

[1] Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona.
[2] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid
[4] Department of Cell Biology, Physiology and Immunology, Universitat de Barcelona, Barcelona, 08028, Spain.
[5] Institute of Neurosciences, University of Barcelona, 08028 Barcelona, Spain.
[6] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, 28029, Spain.
[7] Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, 08028, Spain.
[8] Institut Químic de Sarrià, University Ramon Llull, Barcelona, 08017, Spain.

The hallmark of Lafora disease (LD), a fatal childhood-onset dementia, is the accumulation of the so-called Lafora Bodies (LBs) - in several tissues, including the brain. LBs are composed of poorly-branched glycogen and a number of associated proteins, including p62, an autophagy adaptor that participates in the aggregation and clearance of misfolded proteins. Until recently, it was widely believed that brain LBs were present exclusively in neurons and thus that LD pathology derived exclusively from their accumulation in this cell population. We have demonstrated that LBs are also present in astrocytes, and that impeding LB accumulation specifically in this cell type prevents neuroinflammation, autophagy impairment and metabolic changes characteristic of LD. Furthermore, our results show that p62 participates in the formation of LBs, and that the sequestation of poorly-branched glycogen into LBs is a cellular protective mechanism through which to reduce the deleterious consequences of the accumulation of the polysaccharide in the brain. These results unveil the deleterious consequences of the excessive accumulation of glycogen in astrocytes, which might have implications in other neurodegenerative conditions.

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**NOTES**

## O.5. GREEN TEA AND COCOA: BENEFICIAL EFFECTS ON AGE-ASSOCIATED REGRESSIVE CHANGES IN THE MOUSE NEUROMUSCULAR SYSTEM

**Gras S**[1]*, Blasco A[1]*, Mòdol-Caballero G2, Tarabal O[1], Casanovas A[1], Piedrafita L[1], Gatius A[1], Hernández S[1], Barranco A[3], Das T[4], Rueda R[3], Pereira SL[4], Navarro X[2], Esquerda JE[1], Calderó J[1]

[1] Facultat de Medicina, Universitat de Lleida and Institut de Recerca Biomèdica de Lleida (IRBLleida), Lleida, Spain
[2] Institut de Neurociències, Universitat Autònoma de Barcelona and CIBERNED, Bellaterra, Spain
[3] Abbott Nutrition, Research and Development, Granada, Spain
[4] Abbott Nutrition, Research and Development, Columbus, OH, USA
* These authors contributed equally to this work

Although the major impact of sarcopenia is skeletal muscle wasting, morphological and molecular changes in distinct components of the neuromuscular system, including spinal cord motoneurons (MNs) and neuromuscular junctions (NMJs) have been found to occur at advanced ages; moreover, around aged MNs, it has been observed noticeable microgliosis and astrogliosis (Blasco et al., 2020). We examined the impact of two flavonoid-enriched diets containing either green tea extract (GTE) catechins or cocoa flavanols on age-associated regressive changes in the neuromuscular system of C57BL/6J mice (Gras et al., 2021)). GTE- and cocoa-supplementation significantly improved the survival rate of mice, reduced the proportion of fibers with lipofuscin aggregates and central nuclei, and increased the density of satellite cells and the expression of PGC-1α in skeletal muscles. Both supplements significantly augmented the number of innervated NMJs and their degree of maturity compared to controls. GTE prominently increased the density of VAChT and VGluT2 afferent synapses on MNs, which were lost in control aged spinal cords, whereas cocoa significantly augmented the proportion of VGluT1 afferent synapses on aged MNs. Moreover, GTE reduced aging-associated microgliosis and increased the proportion of neuroprotective microglial phenotypes.

Our data suggest that some plant flavonoids may be beneficial in the nutritional management of age-related deterioration of the neuromuscular system, and that there might be a benefit to combine different flavonoids to counteract these negative effects in the neuromuscular system. These results should be also considered for the management of patients affected of muscular and MN diseases.

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**NOTES**

## O.6. DISCOVERY OF GENES INVOLVED IN THE MICROGLIA-OPCS CROSSTALK DURING DEMYELINATION AND REMYELINATION

**Enrich-Bengoa J**[1,2], Dégano IR[3,4,5], Manich G[2,6], Perálvarez-Marín A[1,2]

[1] Biophysics Unit, Department of Biochemistry and Molecular Biology, School of Medicine, Universitat Autònoma de Barcelona, Bellaterra
[2] Institut de Neurociències, Department of Biochemistry and Molecular Biology, School of Medicine, Universitat Autònoma de Barcelona, Bellaterra
[3] CIBER Cardiovascular Diseases (CIBERCV), Instituto de Salud Carlos III, Madrid
[4] REGICOR Study Group, Cardiovascular Epidemiology and Genetics Group, IMIM (Hospital Del Mar Medical Research Institute), Barcelona
[5] Faculty of Medicine, University of Vic-Central University of Catalonia (UVic-UCC), Vic
[6] Medical Histology Unit, Department of Cell Biology, Physiology and Immunology, School of Medicine, Universitat Autònoma de Barcelona, Bellaterra

Demyelinating disorders such as multiple sclerosis (MS) are characterized by impaired remyelination due to failure of differentiation from oligodendrocyte progenitor cells (OPCs) to mature myelin-forming oligodendrocytes. Several studies indicate that microglia supports OPCs differentiation, however, there are scarce data about the crosstalk between microglia and OPCs. In this study, we aimed to identify the molecules involved in this crosstalk by using a transcriptomic analysis of microarray studies performed in the cuprizone demyelination-remyelination mouse model. We identified the differentially expressed genes (DEGs) in corpus callosum (CC) during late and early demyelination and also during remyelination. DEGs in microglia and OPCs were also identified during late demyelination. Then, we studied the molecular functions associated to these DEGs. Finally, we used an in silico model to identify the microglia ligands that interact with OPC receptors, as well as the OPC target genes during late demyelintion. Our results showed that during demyelination there were 95 DEGs in common between CC and OPCs, 1 DEGs in common between microglia and OPCs, but no common DEGs among all three. Molecular functions of DEGs showed that several functions were related to CNS myelin such as myelin synthesis, axon ensheathment, oligodendrocyte differentiation and cholesterol biosynthesis. Furthermore, we found 47 microglia ligands that may be interacting with 43 OPC receptors and 115 OPC target genes associated with demyelination. These results reveal potential biomarkers that are involved in de-/remyelination, pointing out the importance of microglia-OPCs crosstalk to understand myelination in physiology and pathophysiology.

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**NOTES**

## SESSION 1B

## DEVELOPMENT AND RELATED DISEASES

### O.7. SPECIFIC CONTRIBUTION OF REELIN EXPRESSED BY CAJAL-RETZIUS CELLS OR GABAERGIC INTERNEURONS TO CORTICAL LAMINATION

**Manso Y**[1,2]*, Vílchez-Acosta A[1,2]*, Cárdenas A[3], Elias A[1,2], Pascual M[1,2], Martínez-Losa M[4], Nairn AC [4], Borrell V[3], Soriano E[1,2]

[1] Department of Cell Biology, Physiology and Immunology, Faculty of Biology and Institute of Neurosciences, University of Barcelona, 08028 Barcelona, Spain.
[2] Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), ISCIII, 28031 Madrid, Spain.
[3] Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, 03550 Sant Joan d'Alacant, Spain.
[4] Department of Psychiatry, Yale University School of Medicine, New Haven, 06508 Connecticut, USA
* These authors contributed equally to this work

The extracellular protein Reelin, expressed by Cajal-Retzius cells at early stages of cortical development and at late stages by GABAergic interneurons, regulates radial migration and the “inside-out” pattern of laminar positioning. Current models of Reelin functions in corticogenesis focus on early Cajal-Retzius cell-derived Reelin in layer I. However, neurodevelopmental disorders linked to Reelin deficits, such as Schizophrenia and Autism, are related to GABAergic interneuron-derived Reelin, although its role in migration has not been established. In order to dissect the specific contribution of Reelin from each cellular population to cortical development, we have generated 2 transgenic mouse models with selective inactivation of the RELN gene in either Cajal-Retzius cells or GABAergic interneurons. Our data supports both overlapping and specific functions of Reelin expressed by these two cell populations in distinct features of the reeler phenotype. The present study highlights a fundamental role of GABAergic interneuron-derived Reelin in neuronal migration, in addition to CR cell-expressed Reelin. Furthermore, we observed transient migratory deficits, indicating that Reelin expressed by either neuronal population is sufficient to reverse some lamination defects. On the basis of our findings, we propose a novel model of Reelin action in corticogenesis based on the spatial and cell-specific expression of this key protein. Because several neuropsychiatric disorders are linked to Reelin deficits in interneurons, this study may provide a better understanding of the cellular mechanisms involved in the pathogenesis of human neuropsychiatric disorders linked to abnormal neuronal migration and Reelin deficits.

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**NOTES**

## O.8. THE ROLE OF THE RNA-BINDING PROTEIN STAUFEN 2 DURING NEUROGENESIS

**Fernández Moya SM**[1,2], Gutiérrez-Franco A[1,2], Plass M[1,2,3]

[1] Gene Regulation of Cell Identity, Regenerative Medicine Program, Bellvitge Institute for Biomedical Research(IDIBELL), L'Hospitalet del Llobregat, Barcelona, Spain
[2] Program for Advancing Clinical Translation of Regenerative Medicine of Catalonia, P-CMR[C], L'Hospitalet del Llobregat, Barcelona, Spain
[3] Center for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

Neurogenesis is a crucial process by which new neurons are formed during cortex neurogenesis in the embryo. Still, the brain maintains a limited capacity to regenerate after birth and throughout our lifespan. In humans, this regenerative capacity is linked to adult neurogenesis and alterations of this process are a common hallmark of neurodegenerative diseases such as Alzheimer's disease (AD). Recent studies indicate that posttranscriptional regulation and RNA binding proteins (RBPs) are critical to fine-tune neurogenesis and mediate cortical development. Yet, little is known about the role of these RNA regulatory networks in this process. Staufen 2 (Stau2) is a double-stranded RBP that has been involved in the asymmetric distribution of mRNAs in neuronal stem cells (NSCs), which is critical for NSC maintenance and differentiation in brain development and function. To unravel the RNA regulatory networks controlled by Stau2 at different stages in human neurogenesis, we have established a differentiation protocol of human-induced pluripotent stem cells (hiPSCs) to different neurogenic populations. Using CRISPR-Cas9 we have obtained several heterozygote clones which show a strong reduction in Stau2 mRNA and protein levels. After 11 days of hiPSCs to Neuroepithelial cells differentiation we can observe a clear enrichment of Map2+ and Tuj1+ neuronal populations compared to control cell line. Moreover, mRNA levels of Sox2, Foxg1 and Map2 are altered in the early stages of differentiation. Together, this provides new insight into the functional role of Stau2 in early neurogenesis and its implication in neurodevelopmental- and neurodegenerative diseases.

**NOTES**

## O.9. ARTIFICIAL EXTRACELLULAR MATRIX SCAFFOLDS OF MOBILE MOLECULES ENHANCE MATURATION OF HUMAN STEM CELL-DERIVED NEURONS

**Ortega JA**[1,2], Álvarez Z[3,4,5], Sato K[3,6], Sasselli IR[6], Kolberg-Edelbrock AN[3,7], Quinlan K[8], Mussumeci C[9], Stupp SI[3,4,5,6,7], Kiskinis E[1,3,10]

[1] The Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, USA.
[2] Department of Pathology and Experimental Therapeutics, University of Barcelona, L'Hospitalet de Llobregat, Spain
[3] Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, USA.
[4] Department of Medicine, Northwestern University, Chicago, USA.
[5] Institute of Bioengineering of Catalonia, Barcelona, Spain.
[6] Department of Chemistry, Northwestern University, Evanston, USA.
[7] Department of Biomedical Engineering, Northwestern University, Evanston, USA.
[8] University of Rhode Island, Rhode Island, USA.
[9] Department of Materials Science and Engineering, Northwestern University, Evanston, USA.
[10] Department of Neuroscience, Northwestern University Feinberg School of Medicine, Chicago, USA

Induced pluripotent stem cell (iPSC)-based technologies offer a unique resource for modeling development and disease of the human central nervous system (CNS). However, human iPSC models are still fraught with significant technical limitations including inefficient maturation and reduced long-term viability of neurons. These problems are in part due to a poor recreation of the native extracellular matrix (ECM) in vitro. We hypothesized that establishing a bioactive ECM environment that mimics the adult CNS would facilitate the functional maturation of iPSC-derived neurons. We utilized peptide amphiphiles (PAs) which self-assemble into supramolecular nanofibers that morphologically and chemically mimick the adult CNS ECM. We designed 4 distinct PA-nanofibers containing the bioactive peptide IKVAV found in Laminin-alpha-1, which is higher expressed in the adult CNS and plays a major role in neuronal behavior. The 4 IKVAV-PAs have an almost identical chemical composition, except for a 4 amino acids modification in the non-bioactive domain that makes the IKVAV epitope be displayed in a more or less mobile fashion. Interestingly, proteomic, biochemical and functional assays reveal that scaffolds with highly mobile molecules lead to enhanced beta-1-integrin pathway activation, reduced aggregation, increased arborization, and mature electrophysiological activity of human iPSC-derived neurons. Our work highlights the importance of designing bioactive ECMs to study the development, function and dysfunction of human neurons in vitro.

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**NOTES**

## O.10. INDIRECT PATHWAY LINAGE-SPECIFIC ALTERATIONS FROM EARLY DEVELOPMENT IN HUNTINGTON'S DISEASE ZQ175 EMBRYOS

**Vila C**[1], Esteve-Codina A[2], Londoño F[1], Molina F[1], Canals JM[1]

[1] Laboratory of stem cells and regenerative medicine, Department of biomedical sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona; Creatio - Production and Validation Center of Advanced Therapies, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona; Neuroscience Institute, University of Barcelona, Barcelona; August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona; and Networked Biomedical Research Centre for Neurodegenerative Disorders (CIBERNED).

[2] Centro Nacional de Análisis Genómico (CNAG-CRG) - Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST); and Universitat Pompeu Fabra (UPF), Barcelona, Cataluña, Spain.

Huntington's disease (HD) is a currently incurable neurodegenerative disorder that manifests itself through motor and cognitive symptoms due to a predominant loss of Medium Spiny Neurons (MSNs) in the striatum. Although HD is a neurodegenerative disease, growing evidence show that developmental alterations could be a key factor in the disease, determining the future vulnerability of certain cell types, such as striatal MSNs. Here we show an early HD-related expression pattern elucidated by bulk and 10X single cell RNA-seq performed on wt and HD isolated striatal primordia at different developmental stages. Transcriptomic data together with histological analyses revealed that early alterations in cell cycle progression and cell fate determination in HD embryos give rise to an imbalance between specific neural precursor cells and mature neurons, specifically affecting the indirect MSNs (iMSNs) linage formation. Moreover, iMSNs undergo into specific apoptosis at postnatal stages in HD that produces a reduction of the iMSNs population inhabiting in the adult striatum in HD. Developmental alterations can set the stage for the HD-specific vulnerability of iMSNs and basal ganglia homeostasis loss. Identifying and modulating candidate pathways or genes is crucial to develop new strategies to restore neuronal homeostasis and protect striatal MSNs halting disease progression.

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**NOTES**

## O.11. DISRUPTED IN SCHIZOPHRENIA 1 GENE (DISC1) AS A POTENTIAL MEDIATOR OF DERMATOGLYPHIC NEURODEVELOPMENTAL MARKERS IN SCHIZOPHRENIA

**Sotero-Moreno A**[1], Guardiola-Ripoll[1,2], Moreira M[2], Giralt-López M [2,4], Almodóvar-Payá C[1,3], Hostalet N[1], Miret S[5], Campanera S[5], Muñoz MJ[6], Salvador R[1,3], Fañanás L[3,7], Pomarol-Clotet E[1,2], Fatjó-Vilas M[1,3,5]

[1] FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain
[2] CIBERSAM (Biomedical Research Network in Mental Health; Instituto de Salud Carlos III), Madrid, Spain
[3] Psychiatry Service, Germans Trias i Pujol University Hospital, Badalona, Spain
[4] Psychiatry and Legal Medicine Department, Autonomous University of Barcelona, Cerdanyola del Valles, Spain
[5] Adult Mental Health Centre, Psychiatry, Mental Health and Addictions Service. University Hospital Santa Maria, Lleida, Spain.
[6] Adolescent Psychiatry Unit, Benito Menni Hospital, Sant Boi de Llobregat, Spain.
[7] Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain.

Dermatoglyphic patterns (i.e. palm- and fingerprints) have been consistently associated with schizophrenia (SZ) (Bramon et al., 2005). This relationship stands on the common ectodermal embryonic origin of the epidermis and central nervous system (Babler, 1991). As regards the altered neurodevelopmental trajectories associated with SZ, the DISC1 gene is considered a key factor due to its role in critical neurodevelopment and signalling processes (Tomoda et al., 2017). Beyond previous data on the impact of DISC1 genetic variants on the risk for psychotic disorders (Ma et al., 2018; Palo et al., 2007), we have recently reported two DISC1 haplotypes rs6675281-1000731-rs999710to be associated with the risk and working memory-related brain activity in SZ through a neuroimaging genetics approach (Guardiola-Ripoll et al., 2022). However, little is known about the DISC1 effect on other neurodevelopmental markers. Accordingly, we aimed to test whether DISC1 genetic variability plays a role in the relationship between dermatoglyphics and SZ. In a sample of 61 nuclear families (healthy first-degree relatives and offspring with SZ, n=208), we assessed different dermatoglyphic markers: the total a-b ridge count (TABRC), the fluctuating asymmetry of the a-b ridge count (FA_AB) and the finger pattern intensity (FPI). 7 SNPs at DISC1 were genotyped. We evaluated genetic associations with SZ and dermatoglyphic markers through a TDT/QTDT analysis (UNPHASED). We report the effect of different SNPs and haplotypes at DISC1 gene associated with: i) reduced pattern intensity scores (i.e. less complex finger figures), ii) an increase of FA_AB (considered a marker of developmental instability). These results suggest the role of DISC1 gene in the development and variability of both SZ and dermatoglyphic patterns and reinforce its involvement in neurodevelopmental processes underlying the aetiology of SZ. The identified associations encourage the combined use of genetics and dermatoglyphics to assess neurodevelopmental alterations predisposing to SZ.

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**NOTES**

## O.12. UNRAVELLING THE ENDOPHENOTYPIC CHARACTERISTICS OF GENERATED GRIN-RELATED DISORDERS ZEBRAFISH MODELS

**Locubiche Serra S**[1,2], Olivella M[3], De Santis F[1], Altafaj X[2], Rubbini D[1], Terriente J[1]

[1] ZeClinics, Institut de Recerca Germans Trias I Pujol (IGTP), Badalona, Spain

[2] Medical School, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain

[3] Universitat de Vic-Universitat Central de Catalunya, Vic, Spain

NMDA-type ionotropic glutamate receptors play pivotal roles in synaptic development, plasticity, neural survival, and cognition. Recent advances on Next-Generation Sequencing revealed the association of de novo mutations affecting GRIN genes (encoding GluN subunits of the NMDAR) with neurodevelopmental disorders, so-called GRIN-related disorders (GRD). GRD is a rare condition with a clinical spectrum dictated by both the affected GRIN gene and the functional outcomes of the mutated residue/s, primarily affecting glutamatergic neurotransmission and causing synaptopathies. Accordingly, generation of an in vivo library is required to delineate the neurological alterations and ultimately to identify personalized therapeutic approaches for GRDs. In the context of GRD, zebrafish appear as an optimal animal model, since it provides several advantages from biomedical and industrial points of view.

To address this objective, CRISPR-Cas9-based genome editing technology has been applied for the obtention of knockout models of Zebrafish paralogous GRIN1, GRIN2A and GRIN2B genes. Single mutant larvae showed no effect on survival rate, and allowed to define the spatio-temporal expression pattern of grin genes in larval stages. Phenotypic assessments have been performed in pharmacological acute GRD models, revealing the presence of both behavioral and motor phenotypes, allowing the optimization of the behavioral paradigms of interest. Currently, the proposed procedure is being used to evaluate the generated GRD models. In the short term, the comprehensive phenotyping of Zebra-GRIN models will allow to define GRD-like alterations and, importantly, to evaluate the therapeutic efficacy of repurposed and EMA-approved putative NMDAR allosteric modulators, to ultimately allow personalized therapies for GRD patients.

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**NOTES**

## SESSION 2A

## NEURODEGENERATIVE DISEASES

### O.13. SINGLE-CELL TRANSCRIPTOMICS OF IPSC-DERIVED NEURONS REVEALS FUNCTIONAL CHANGES IN ALZHEIMER'S DISEASE

**Gutiérrez-Franco A**[1,2], Hassan M[1,2], Ake F[1,2], Fernández-Moya S[1,2], Plass M*[1,2,3]

[1] Gene Regulation of Cell Identity, Regenerative Medicine Program, Bellvitge Institute for Biomedical Research (IDIBELL), 08908, L'Hospitalet del Llobregat, Barcelona, Spain
[2] Programfor Advancing Clinical Translation of Regenerative Medicine of Catalonia, P-CMR[C], 08908, L'Hospitalet del Llobregat, Barcelona, Spain
[3] Center for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 28029, Madrid, Spain

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease that heavily burdens healthcare systems worldwide. Most AD patients are sporadic and despite all the efforts, we still do not know the molecular mechanisms triggering the development of AD. One of the main problems to study AD and other neurodegenerative diseases is the lack of good experimental models that recapitulate the pathological features of the disease. In that context, induced pluripotent stem cell (iPSC) technology has provided an excellent tool to modeldisease pathogenesis considering the patients' genetic background.

In this project, we have used single-cell transcriptomics (scRNA-seq) to study the molecular changes that happen during the differentiation of iPSCs derived from sporadic AD patients to neurons. Preliminary results show that AD-derived neural progenitor cells already show changesin the expression of genes previously associated with AD or related to neuronal differentiation and RNA processing. These results demonstrate that neurons from sporadic AD patients show transcriptomic differences before the onset of the disease and thus can be used as a relevant model to study the molecular networks driving AD. Future work will be directed to validate these findings and assess its impact in the development of AD.

Taken together, our results show that the combination of iPSC technology and scRNA-seq is a potent tool for the study of the molecular mechanisms triggering the development of neurodegenerative diseases such as AD.

**NOTES**

## O.14. DIFFERENTIAL EFFECTS OF AMYLOID-β AND TAU NEUROPATHOLOGY ON COGNITIVE AND EMOTIONAL SYMPTOMS IN NOVEL ALZHEIMER'S DISEASE TRANSGENIC MICE

**Capilla-López MD**[1,2], Deprada A[1,2], Martínez-Gallego I[4], Andrade-Talavera Y[4], Puigdellívol M[1,3], del Ser-Badia A[1,2], Rodríguez-Alvarez J[1,2], Rodríguez-Moreno A[4], Parra-Damas A[1, 2], Saura CA[1,2]

[1] Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain.
[2] Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.
[3] Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain.
[4] Department of Physiology, Anatomy and Cell Biology, Universidad Pablo de Olavide, Sevilla, Spain

Amyloid plaques and neurofibrillary tangles containing amyloid-β (Aβ) peptides and hyperphosphorylated tau protein, respectively, are the neuropathological hallmarks of Alzheimer's disease (AD). However, how concurrent Aβ and tau pathologies synergize to disrupt specific brain circuits during AD progression is not fully understood. Here, we characterize novel double transgenic mice expressing mutant human amyloid precursor protein (APP) and microtubule-associated protein tau (Tau) in excitatory neurons, to evaluate age, sex, and pathological effects on brain circuitry and behavior. We detected transgene effects, but not gender differences, on neuropathological progression and cognitive and emotional deficits. Interestingly, tau phosphorylation and aggregation were increased in the hippocampus and amygdala of double APP/Tau mice compared to single mutant Tau mice, whereas Aβ pathology was not affected by mutant Tau expression. Moreover, adult APP and APP/Tau, but not Tau mice, exhibit anxious behavior and impaired fear memory extinction associated with increased Aβ, but not tau pathology, in limbic regions. Tissue clearing, confocal imaging and electrophysiological recordings reveal that memory deficits coincide with impaired activation of excitatory neurons, synaptic accumulation of tau and reduced presynaptic proteins in the hippocampus of APP/Tau mice. Our results indicate that the progression and interaction of Aβ and tau pathologies in specific brain circuits exacerbates synaptic pathology and memory and emotional symptoms in AD.

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**NOTES**

## O.15. IS RTP801/REDD1 INVOLVED IN tRNA PROCESSING ?

**Campoy-Campos G** [1,2,3], Torres AG [4,5], Solana-Balaguer J [1,2,3], Pérez-Sisqués L [1,2,3,6], Alberch J [1,2,3,7], Pérez-Navarro E [1,2,3,7], Ribas de Pouplana L [4,5,8] , Malagelada C[1,2,3]

[1] Universitat de Barcelona, Facultat de Medicina i Ciències de la Salut, Departament de Biomedicina, Unitat de Bioquímica, Barcelona, Catalonia, Spain
[2] Institut de Neurociències, Barcelona, Catalonia, Spain
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain
[4] Institut de Recerca Biomèdica (IRB Barcelona), Barcelona, Catalonia, Spain
[5] Barcelona Institute of Science and Technology (BIST), Barcelona, Catalonia, Spain
[6] Centre for Craniofacial and Regenerative Biology, King's College London, London, United Kingdom
[7] IDIBAPS-Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Catalonia, Spain
[8] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain

Introduction: RTP801/REDD1 is a stress responsive protein overexpressed in neurons of patients with neurodegenerative disorders such as Parkinson's and Huntington's diseases. Its main function is to inhibit the mTOR pathway, but, if this inactivation is sustained in time, it has a pro-apoptotic effect in differentiated cells like neurons. Nevertheless, RTP801 might have other functions not yet elucidated. In preliminary proteomic studies from our laboratory, RTP801 was found to interact with HSPC117 and DDX1, two proteins that are part of the tRNA splicing ligase complex, which performs the ligation of the tRNA fragments generated during splicing.

Aims: Since alterations in tRNA metabolism have recently been associated to the development of some neurodegenerative diseases, we aimed to deeper study the relationship between RTP801 and these tRNA-processing enzymes.

Results: Here, we confirm by immunoprecipitation that endogenous RTP801 interacts with the tRNA splicing ligase complex, concretely with DDX1, HSPC117 and CGI-99. We also observe changes in the cellular distribution of these tRNA-processing enzymes when we modify the levels of RTP801. Additionally, the maturation of intron-containing tRNAs is altered in the cortex of 2-month-old RTP801 knockout mice compared to wild-type. Finally, we also observe related alterations in hippocampal and striatal postmortem samples from patients with Alzheimer's and Huntington's diseases, where RTP801 is involved in the pathogenesis.

Conclusions: These results suggest a novel role of RTP801 in tRNA processing, which must be further studied, as RTP801 could be a potential target to prevent altered tRNA metabolism in neurodegenerative diseases.

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**NOTES**

## O.16 AN EXTRACELLULAR SMALL RNA BIOSIGNATURE IN PLASMA IDENTIFIES PREMANIFEST HUNTINGTON´S DISEASE

**Herrero-Lorenzo M**[1], Pérez J[3,4,5], Escaramís G[1,2], Pérez-González R[3,4,5], Kulisevsky J[3,4,5], Gámez-Valero A[1,2]*, Martí E[1,2]*

[1] Department of Biomedicine, Faculty of Medicine, Institute of Neurosciences, University of Barcelona, C/ Casanova 143 (08036) Barcelona, Spain
[2] Biomedical Research Networking Center for Epidemiology and Public Health (CIBERESP), Spanish Ministry of Science and Innovation, Madrid, Spain
[3] Movement Disorders Unit, Neurology Department, Sant Pau Hospital, Barcelona, Spain
[4] Biomedical Research Institute (IIB-Sant Pau), Barcelona, Spain
[5] Center for Networked Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain

Despite the advances in the understanding of Huntington's disease (HD), no disease-modifying treatments exist, and therapeutic development and HD-clinical trials continue to fail. Major efforts are being invested in the assessment of measurable outcomes in early diagnosis and prognosis for optimal therapeutic response. Recent insights on Huntington's disease have pointed to a profound role of RNA in the neuropathogenesis of the disorder. Specifically, growing evidence indicates that small non-coding RNA (sRNA) are key players in the disease. The profiling of extracellular sRNAs (exRNA), found in body fluids as freely circulating, associated to protein-complexes, and/or encapsulated in extracellular vesicles (EVs), supposes a promising approach for defining non-invasive biomarkers that reflect disease status. Using an optimal method for plasma sub-fractionation and EVs purification by Size-exclusion chromatography (SEC) and Ultrafiltration (UF), we explored sRNA content in EVs and Non-EVs compartments, providing a deep exRNA analysis and offering a complementary source of valuable information. Characterization of plasma-EVs from three different cohorts, including healthy controls, premanifest HD, and manifest HD, revealed no differences in size and morphology of EVs. Using SeqCluster bioinformatic tool for sRNA annotation and quantification, we highlighted that most differentially expressed sRNAs in HD-EVs are downregulated in comparison to Control-EVs, with many changes occurring at premanifest stages. Those sRNAs showing the most differential profile between groups were validated as potential future biomarkers for HD. These findings suggest that alterations in circulating exRNAs may reflect early clinical and pathological changes in HD patients.

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**NOTES**

## O.17. IN VIVO REDUCTION OF AGE-DEPENDENT NEUROMELANIN ACCUMULATION MITIGATES FEATURES OF PARKINSON'S DISEASE

**Compte J**[1], Gonzalez-Sepulveda M[1], Cuadros T[1], Parent A[1], Nicolau A[1], Guillard-Sirieix C[1], Peñuelas N[1], Romero-Giménez J[1], Lorente-Picon M[1], Laguna A[1], Vila M[1,2,3]

[1] Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute (VHIR)-Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain
[2] Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, Spain
[3] Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Humans accumulate with age the dark-brown pigment neuromelanin inside specific neuronal groups. Neurons with the highest neuromelanin levels are particularly susceptible to degeneration in Parkinson's disease, especially dopaminergic neurons of the substantia nigra (SN), the loss of which leads to characteristic motor Parkinson's disease symptoms. In contrast to humans, neuromelanin does not appear spontaneously in most animals, including rodents, and Parkinson's disease is an exclusively human condition. Using humanized neuromelanin-producing rodents, we recently found that neuromelanin can trigger Parkinson's disease pathology when accumulated above a specific pathogenic threshold. Here, by taking advantage of this newly developed animal model, we assessed whether the intracellular buildup of neuromelanin that occurs with age can be slowed down in vivo to prevent or attenuate Parkinson's disease. Because neuromelanin derives from the oxidation of free cytosolic dopamine, we enhanced dopamine vesicular encapsulation in the SN of neuromelanin-producing rats byviral vector-mediated overexpression of vesicular monoamine transporter 2 (VMAT2). This strategy reduced the formation of potentially toxic oxidized dopamine species that can convert into neuromelanin and maintained intracellular neuromelanin levels below its pathogenic threshold.
Decreased neuromelanin production was associated with an attenuation of Lewy body-like inclusion formation and a long-term preservation of dopamine homeostasis, nigrostriatal neuronal integrity andmotor function in these animals. Our results demonstrate the feasibility and therapeutic potential of modulating age-dependent intracellular neuromelanin production in vivo, thereby opening an unexplored path for the treatment of Parkinson's disease and, in a broader sense, brain aging.

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## O.18 THE Y172-RELATED PROTEIN: A NEW ACTOR IN MOTONEURON PHYSIOPATHOLOGY

**Gatius A**, Garcia-Segura P, Tarabal O, Cayuela A, Garcerá A, Casanovas A, Salvany S, Gras S, Blasco A, Piedrafita L, Hernández S, Soler RM, Esquerda JE, Calderó J

[1] Departament de Medicina Experimental, Facultat de Medicina, Universitat de Lleida and Institut de Recerca Biomèdica de Lleida (IRBLleida), Lleida

Motor behavior is mediated by a highly coordinated activity of motoneurons (MNs). The excitation state of MNs is controlled by different synaptic afferents, including cholinergic synapses (C-boutons). Alterations in C-boutons appear to play an important role in MN diseases, particularly in amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). We have previously found that a monoclonal antibody against phospho-c-Jun (serine[Ser63]) – the Y172 antibody – displayed an unexpected labeling in the soma and proximal dendrites of spinal cord MNs. We further analyzed the cytoplasmic Y172-immunostaining in spinal cord MNs of CD1 mice and in mouse models of SMA (Smn2B/-) and ALS (SOD1G93A) and extended the study to the sciatic nerve. In adult spinal cord, MNs displayed strong Y172 immunostaining in the subsurface cistern of C-boutons, but not in other synapse types. In MNs after peripheral nerve transection, and in those from ALS and SMA mice, the Y172 immunostaining associated to C-boutons was significantly reduced, even before the occurrence of cholinergic deafferentation. Studies in the adult sciatic nerve revealed the presence of Y172-positive vesicle-like structures in the cytoplasm of SCs ensheathing MN axons. Other antibodies against either c-Jun or its phosphorylated forms were not able to replicate the same Y172-immunocytochemical pattern, suggesting that the immunoreactivity found in C-boutons and in the cytoplasm of SCs is restricted to the Y172 antibody and could belong to a protein other than c-Jun. Overall, we show a novel unidentified molecular component of the C-bouton organization, which expression is lost in damaged MNs. Moreover, the presence of Y172 in SCs suggests that this protein may play an important role in MN maintenance. Our results lay the foundation for further identifying the Y172-related protein and determining its role in the development, maintenance, plasticity and pathology of MNs.

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**NOTES**

## SESSION 2B

## NEUROTRANSMITTER RECEPTORS AND NEUROPHARMACOLOGY

### O.19 POSITION MATTERS: A DIFFERENTIAL MODULATION OF AMPARs DEPENDING ON THE AUXILIARY SUBUNIT LOCATION INTO THE RECEPTOR COMPLEX

Miguez-Cabello F[1], Castellanos A[1], Picañol X[1], Gratacòs-Batlle E[1], Gasull X[1,2], **Soto D[1,2]**

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute of Neurosciences, Universitat de Barcelona, 08036 Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain

Glutamate is the main neurotransmitter in the brain. In neuronal communication, AMPA receptors (AMPARs) mediate roughly the 90‐95% of fast‐excitatory glutamatergic transmission at synapses. The biophysical properties of these glutamate‐gated ion channels are modulated by several other membrane‐spanning proteins being the members of the Transmembrane AMPAR Regulatory Proteins (TARPs) family the most abundant and studied. Despite the extensive work describing that TARPs modulate many gating and pharmacological properties of AMPARs, it is still unknown how many TARPs are needed to induce significant changes in these intrinsic properties. In this work we described a putative functional stoichiometry in cerebellar granule cells (CGCs) which express a limited variety of AMPAR subunits (only GluA2 and GluA4c) and TARPs (gamma2 and gamma7).

We first tested, in a heterologous expression system (tsA201 cells), different possible stoichiometries present in CGCs by means of electrophysiological recordings. We took advantage of AMPAR:TARP fusion proteins to obtain fixed stoichiometries of 0, 2 or 4 TARPs per AMPAR. Our data surprisingly shows that TARP gamma2 differently modulates biophysical properties of the channel depending on the subunit where it is bound (GluA2 or GluA4). Moreover, we found a 2 TARP per AMPAR (bound to GluA2) stoichiometry in CGCs by comparing data obtained in cell lines with CGCs. Given the huge variability of AMPARs (6 different AMPAR subunits and 6 TARPs – plus several other auxiliary subunits), these results will help to better understand the complexity in the regulation of this key player of synaptic transmission. Moreover, it has been recently described that some drugs affect selectively certain AMPARs depending on the TARP present into the AMPAR complex. TARPs and AMPAR subunits are differentially expressed in the brain and targeting specific populations will undoubtedly reduce broad action drugs side effects. Our work adds a pinch of valuable information for future therapies.

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**NOTES**

## O.20. COMPREHENSIVE DELINEATION AND PRECISION MEDICINE OF GRIN-RELATED NEURODEVELOPMENTAL DISORDERS, A PRIMARY DISTURBANCE OF THE NMDA RECEPTOR

**Altafaj X**[1], Santos-Gómez A[1], Locubiche Serra S[1,2], Soto D[1], Olivella M[3]

[1] Medical School, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain
[2] ZeClinics, Institut de Recerca Germans Trias I Pujol (IGTP), Badalona, Spain
[3] Universitat de Vic – Universitat Central de Catalunya, Vic, Spain

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. Accordingly, 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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## O.21. REMOTE LOCAL PHOTOACTIVATION OF MORPHINE PRODUCES ANALGESIA WITHOUT OPIOID-RELATED ADVERSE EFFECTS

**López-Cano M**[1,2], Font J[3,4], Aso E[1,2], Sahlholm K[1,2,5,6], Cabré G[7], Giraldo J[8,9,10], De Koninck Y[11,12], Hernando J[7], Llebaria A[3], Fernández-Dueñas V[1,2], Ciruela F[1,2]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, School of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain.
[2] Neuropharmacology & Pain Group, Neuroscience Program, Bellvitge Institute for Biomedical Research, IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.
[3] MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain.
[4] Institut de Génomique Fonctionnelle (IGF), University of Montpellier, CNRS, INSERM, Montpellier, France.
[5] Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.
[6] Department of Integrative Medical Biology, Umeå University, Umeå, Sweden.
[7] Departament de Química, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.
[8] Laboratory of Molecular Neuropharmacology and Bioinformatics, Unitat de Bioestadística and Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Spain.
[9] Unitat de Neurociència Traslacional, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT), Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain.
[10] Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Barcelona, Spain.
[11] Institut Universitaire en Santé Mentale de Québec, Québec, Quebec, Canada.
[12] Department of Psychiatry and Neuroscience, Université Laval, Québec, Quebec, Canada.

Background and purpose: Opioid-based drugs are the gold standard medicines for pain relief. However, tolerance and several side effects (i.e. constipation and dependence) may occur upon chronic opioid administration. Photopharmacology is a promising approach to improve the benefit/risk profiles of these drugs. Thus, opioids can be locally activated with high spatiotemporal resolution, potentially minimizing systemic-mediated adverse effects. Here, we aimed at developing a morphine photo-derivative (photocaged morphine), which can be activated upon light irradiation both in vitro and in vivo.
Experimental approach: Light-dependent activity of pc-morphine was assessed in cell-based assays (intracellular calcium accumulation and electrophysiology) and in mice (formalin animal model of pain). In addition, tolerance, constipation and dependence were investigated in vivo using experimental paradigms.
Key results: In mice, pc-morphine was able to elicit antinociceptive effects, both using external light-irradiation (hind paw) and spinal cord implanted fibre-optics. In addition, remote morphine photoactivation was devoid of common systemic opioid-related undesired effects, namely, constipation, tolerance to the analgesic effects, rewarding effects and naloxone-induced withdrawal.
Conclusion and implications: Light-dependent opioid-based drugs may allow effective analgesia without the occurrence of tolerance or the associated and severe opioid-related undesired effects.

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**NOTES**

## O.22. EXPERIMENTAL AND COMPUTATIONAL ANALYSIS OF BIASED AGONISM ON FULL-LENGTH AND A C-TERMINALLY TRUNCATED ADENOSINE A2A RECEPTOR

**Reyes-Resina I**[1,2], Navarro G[3,2 *], Gonzalez A[4,6], Campanacci S[1,6], Rivas-Santisteban R [1,3], Casajuana-Martin N[4], Cordomí A[4], Pardo L [4,6], Franco R[1,2,5 *]

[1] Dept. Biochemistry and Molecular Biomedicine. School of Biology. Universitat de Barcelona. Barcelona. Spain
[2] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas. Instituto de Salud Carlos III, Madrid, Spain
[3] Dept. Biochemistry and Physiology, Faculty of Pharmacy and Food Science. Universitat de Barcelona. Barcelona, Spain
[4] Laboratori de Medicina Computacional, Unitat de Bioestadi´stica, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[5] School of Chemistry. Universitat de Barcelona. Barcelona. Spain
[*] Equal contribution

Biased agonism, the ability of agonists to differentially activate downstream signaling pathways by stabilizing specific receptor conformations, is a key issue for G protein-coupled receptor (GPCR) signaling. The C-terminal domain might influence this functional selectivity of GPCRs as it engages G proteins, GPCR kinases, b-arrestins, and several other proteins. Thus, the aim of this paper is to compare the agonist-dependent selectivity for intracellular pathways in a heterologous system expressing the fulllength (A2AR) and a C-tail truncated (A2AD40R lacking the last 40 amino acids) adenosine A2A receptor, a GPCR that is already targeted in Parkinson's disease using a first-in-class drug. Experimental data such as ligand binding, cAMP production, b-arrestin recruitment, ERK1/2 phosphorylation and dynamic mass redistribution assays, which correspond to different aspects of signal transduction, were measured upon the action of structurally diverse compounds (the agonists adenosine, NECA, CGS-21680, PSB-0777 and LUF-5834 and the SCH-58261 antagonist) in cells expressing A2AR and A2AD40R. The results show that taking cAMP levels and the endogenous adenosine agonist as references, the main difference in bias was obtained with PSB-0777 and LUF-5834. The C-terminus is dispensable for both G-protein and barrestin recruitment and also for MAPK activation. Unrestrained molecular dynamics simulations, at the ls timescale, were used to understand the structural arrangements of the binding cavity, triggered by these chemically different agonists, facilitating G protein binding with different efficacy.

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**NOTES**

## O.23. SCREENING OF ADENOSINE A1 RECEPTOR LIGANDS AS HETEROMER-SELECTIVE LIGANDS FOR THE ADENOSINE A1-DOPAMINE D1 RECEPTOR OLIGOMER

**Llopart N**[1,2] , Alonso A[1,2], Casadó-Anguera V[1,2] , Moreno E[1,2] , Casadó V[1,2]

[1] Molecular Neuropharmacology Group, Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, University of Barcelona, Barcelona, Spain
[2] Institute of Biomedicine of the University of Barcelona, University of Barcelona, Barcelona, Spain

Movement-associated diseases such as restless leg syndrome (RLS) or spinal cord injuries (SCI) occur because of damage or malfunction of the components of the central nervous system (brain and spinal cord). A common hallmark in these pathologies is a dysfunction of the dopaminergic and adenosinergic systems. Dopamine and adenosine receptors, as other G-protein coupled receptors (GPCRs), form homo- and hetero-oligomers. These oligomers show pharmacological and functional properties different from those of the constituent monomers. Evidence has shown the existence of heteromers between adenosine A1 and dopamine D1 receptors (A1R-D1R), in which A1R activation negatively modulates D1R.

Using mammalian cells transfected with A1R or with A1R and D1R, we determined the affinity by radioligand binding assays, and the potency and efficacy, by complemented donor-acceptor resonance energy transfer (CODA-RET) experiments of a library of commercially available A1R agonists and antagonists. Furthermore, we analysed the heteromer's fingerprint (cross-talk and cross-antagonism) related to intracellular signalling, such as intracellular cAMP levels. Overall, we identified the agonists R-PIA, CCPA and GR79236, and the antagonists PSB36, DPCPX, KW3902, SLV320 and theophylline as A1R-D1R heteromer-selective ligands. R-PIA was the agonist with the highest capability to perform negative cross-talk, and theophylline was the antagonist with the lowest ability to exert cross-antagonism. The discovery of A1R-D1R heteromer-selective A1R ligands which potentiate or inhibit the D1R function would allow selecting lead-compounds for the design and synthesis of A1R agonists or antagonists with potential therapeutic use in motor pathologies with fewer side effects.

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## O.24. NUTRIENT-MEDIATED REGULATION OF GLUA1 SURFACE LEVELS

**Rojas R**[1], Fadó [R1], Miñano A[2,4], Rodríguez J[2,4,5], Casals N[1,3]

[1] Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya (UIC), Sant Cugat del Vallès, Spain
[2] Institute of Neurosciences (INc), Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain
[3] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[4] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain
[5] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, New York, NY 10461, USA

It is widely known that brain needs a lot of energy to carry out all its functions. Neurons use most of this energy to maintain glutamatergic synapsis in which AMPA receptors play an important role. Our group hasrecently demonstrated that the basal transport of AMPAR subunit GluA1 to the plasma membrane is downregulated upon glucose depletion. Moreover, it has been described that nutrients modulate synapticstrength, as well as some diets have an impact on learning and memory processes. The aim of my thesis is to elucidate the molecular mechanisms by which different nutrients can regulate synaptic function, analyzing its effects on AMPAR trafficking. To address this objective, primary cortical mouse neurons weretreated with different fatty acids or ketone bodies at 14-15 days of culture. Then, GluA1 surface levels were analyzed by immunocytochemistry after 2 h or 24 h of treatment. Our results indicated that, on theone hand, palmitic acid, a saturated fatty acid, decreased the amount of GluA1 surface levels, while oleic acid and ω-3 docosahexaenoic acid, two unsaturated fatty acids, increased theamount of GluA1 subunit at plasma membrane. On the other hand, β-hydroxybutyrate, a ketone body used as a source of energy inthe brain during ketogenic diet (based on low carbohydrate and high fat intake), raised GluA1 surface levelsat short and long times. We are now unravelling the role of PI4P in this regulation. In summary, we demonstrate that saturated fatty acids reduce GluA1 trafficking, while polyunsaturated fatty acids and ketone bodies seem to have beneficial effects in neurons. These results give insight into why certain dietsare able to delay cognitive impairment in neurodegenerative diseases.

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**NOTES**

## SESSION 3A

## NEURONAL CIRCUITS AND BRAIN PLASTICITY

### 0.25. YOUNG NEURONS FOR YOUR OLD CORTEX

Coviello S[1], Gramuntell Y[1], Klimczak P[1,2], Varea E[1], Blasco-Ibañez JM[1], Crespo C[1], Gutierrez[3], **Nacher J**[1,2,4]

[1] Neurobiology Unit, Program in Neurosciences and Institute of Biotechnology and Biomedicine (BIOTECMED), Universitat de València, Burjassot, Spain
[2] Spanish National Network for Research in Mental Health, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain
[3] Unidad de Cirugía de la Epilepsia, Hospital Universitario La Fe, Valencia, Spain. 4 Fundación Investigación Hospital Clínico de Valencia (INCLIVA), Valencia, Spain

Studying the brains of adult rodents, we and others realized that, in addition to the classical neurogenic regions, cells expressing molecules of immature neurons could also be observed in the olfactory cortex. We analyzed this cell population and concluded that they were neurons that had just begun to establish synaptic connections and were mostly isolated from neuronal circuits. Interestingly, these neurons were produced during embryonic life and remained in an immature state during adult life. Since their number was greatly reduced as the animals aged, it would be possible that they were progressively integrated in the circuitry. Indeed, using transgenic mice, we have recently shown that they progressively integrate as functional excitatory neurons. These immature neurons can be found in the cerebral cortex of practically all taxonomic groups of mammals. As we ascend in the evolutionary scale and the complexity of the cerebral cortex, these immature neurons have a broader distribution and are not restricted only to the olfactory cortex. Using surgical samples from epileptic patients and post-mortem tissue, we have found cells with different levels of dendritic complexity expressing immature neuronal markers in the human cerebral cortex. These immature cells belonged to the excitatory lineage. The most developed cells had some puncta expressing inhibitory and excitatory synaptic markers apposed to their perisomatic and peridendritic regions and ultrastructural analysis suggest the presence of synaptic contacts. These cells did not present glial cell markers, although astroglial and microglial processes were found in close apposition to their somata and dendrites. The presence of these immature neurons in the adult human cortex opens a wide repertoire of questions regarding their integration, their function and their putative potential as a “reservoir” of young plastic neurons, which under physiological or pathological circumstances may complete their differentiation program to be recruited into preexisting neural circuits.

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**NOTES**

## O.26. A SYSTEMS BIOLOGY APPROACH TO DECIPHER THE SYNAPTIC MOLECULAR ALTERATIONS UNDERPINNING THE INTELLECTUAL DISABILITY CAUSED BY SYNGAP1

Gou G[1,2], Reig-Viader R[1,2], del Casillo D[1,2], Peñuela A[1,2], Rumbaugh G[3], **Bayés A**[1,2]

[1] Molecular Physiology of the Synapse Laboratory, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain.
[2] Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain
[3] Department of Neuroscience, The Scripps Research Institute, Jupiter, United States

Forebrain postsynaptic densities (PSDs), found in dendritic spines, express more than 2,000 different proteins, endowing neurons with the synaptic plasticity mechanisms required for cognition and behaviour. In mammals the Syngap1/SYNGAP1 gene encodes for one of the more abundant PSD proteins, SynGAP, which has a key role in repressing small GTPases signalling at dendritic spines. Loss of function mutations in the SYNGAP1 gene cause autosomal mental retardation type five (MRD5, OMIM), which is characterized by intellectual disability (ID) and epilepsy, as well as autistic traits in approximately half of the affected individuals, among other impairments. It is estimated that this form of intellectual disability could represent up to 1% of all non-syndromic forms of ID. In this study we characterise the proteomic alterations derived from Syngap1 haploinsufficiency in the hippocampal PSD. The rescue of these alterations, as well as the effect of SynGAP ablation were also investigated in two conditional Syngap1+/- mouse lines. Importantly, we found that over 10% of the PSD proteome has abnormal expression levels due to SynGAP reduced levels. These analyses revealed that proteins related to small GTPases, translation and energy production, among others, were significantly altered in Syngap1+/- mice. In addition, ~83% of the alterations observed could be recovered after normal SynGAP levels were genetically restored at PND21. Based on gene set enrichment analyses, the molecular alterations observed after SynGAP rescue would be compatible with a less clinically severe scenario. Lastly, the induction of SynGAP deficit at PND21 resulted in a similar number of altered proteins as in embryonic Syngap1 haploinsufficiency, yet these two scenarios shared few affected proteins, suggesting a developmental role in the molecular alterations observed in the embryonic deficit of SynGAP. Overall our findings reveal the far-reaching implications that SynGAP deficiency has at the molecular level and identifies potential molecular targets for pharmacological intervention.

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**NOTES**

## O.27. ROLE OF NEURON-DERIVED EXTRACELLULAR VESICLES IN SYNAPTIC PLASTICITY

**Solana Balaguer J** [1,2,3], Coll Manzano A [1,2,3,4], Campoy Campos G [1,2,3], Pérez Sisqués L [1,2,3,5], Martín Flores N [1,2,3,6], Alberch J [1,2,3,4], Soriano J [7,8], Masana M [1,2,3,4], Malagelada C [1,2,3]

[1] Institut de Neurociències, School of Medicine and Health Sciences, Barcelona, Spain
[2] Universitat de Barcelona, Facultat de Medicina i Ciències de la Salut, Departament de Biomedicina, Unitat de Bioquímica, Barcelona, Catalonia, Spain
[3] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, (CIBERNED), Spain
[4] Institut d'Investigacions Biomèdiques August Pi i Sunyer, (IDIBAPS), Barcelona, Spain
[5] King's College London, Centre for Craniofacial and Regenerative, London, United Kingdom
[6] University College London, Cell and Developmental Biology, London, United Kingdom
[7] Universitat de Barcelona, Física de la Matèria Condensada, Barcelona, Spain
[8] Universitat de Barcelona, Institute of Complex Systems (UBICS), Barcelona, Spain

Neurodegenerative diseases induce an impairment in synaptic plasticity which eventually leads to cognitive symptoms in patients. Extracellular vesicles (EVs), which are involved in intercellular communication, have been suggested to be involved in synaptic processes, as they are carriers of bioactive miRNAs, proteins and lipids that can influence firing rate in recipient neurons. The aim of this study is to investigate whether neuronal EVs have a direct role in the regulation of synaptic plasticity. Extracellular vesicles were isolated from rat cortical neurons culture media, by ultracentrifugation, and used to treat sister cultures for 24h. Samples were subjected to immunohistochemistry, Western blotting and calcium imaging analysis. We described that EVs are taken up by neurons both in the soma and in dendrites, and even in synaptic spines. We found that neuronal EVs carry synaptic proteins and enhance the consolidation of glutamatergic synapses in recipient neurons. We also observed a mild effect of EVs over neural network dynamics. Moreover, EVs had a trophic effect in neurons under nutrient deprivation conditions. All these data put neuronal EVs in the spotlight to understand synaptic plasticity impairment in neurodegenerative conditions and to use them as a possible therapeutic approach.

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**NOTES**

## O.28. CRTC1 REGULATES NEURONAL ACTIVITY-DEPENDENT GENE PROGRAMS MEDIATING SYNAPTIC PLASTICITY AND NEURONAL EXCITABILITY

**Parra-Damas A**[1,2], del Ser-Badia A[1,2], Deprada A[1,2], Rodríguez-Álvarez J[1,2], Saura CA[1,2]

[1] Institut de Neurociències, Department de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra.
[2] Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED).

Long-term synaptic plasticity and memory requires neuronal activity-induced gene expression regulated by the transcription factor cAMP-response element binding protein (CREB). CREB-dependent gene expression requires transcriptional coactivators, such as CREB binding protein (CBP/P300) and CREB-regulated transcription coactivator-1 (CRTC1). However, the specific gene programs regulated by CRTC1 during induction of neuronal activity remain unknown. Here, we used chromatin immunoprecipitation sequencing (ChIP-seq) to analyze the genome-wide occupancy profiles of CRTC1 in cultured neurons in both basal and stimulated conditions after treatment with forskolin and potassium chloride (FSK/KCl), which induces CRTC1 dephosphorylation, nuclear translocation and binding to promoter regions of target genes. We detected strong induction of CRTC1 occupancy in a subset of CREB target genes upon FSK/KCl treatment, including proximal promoter regions and distal regions comprising well-characterized neuronal activity-regulated enhancers. Interestingly, we identified activity-dependent binding of CRTC1 at genes mediating neuronal excitability and synaptic plasticity, including neurotransmitter receptors, synaptic proteins and transcriptional regulators. These results suggest that CRTC1 regulates gene programs mediating not only local synaptic plasticity, but also global neuronal excitability.

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**NOTES**

## O.29. HYPOTHALAMIC PREGNENOLONE MEDIATES RECOGNITION MEMORY IN THE CONTEXT OF METABOLIC DISORDERS

**Ramírez S**[1], Haddad-Tóvolli R[1], Radosevic M[1], Claret M[1,3,4]

[1] Neuronal Control of Metabolism (NeuCoMe) Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.
[2] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (ciberdem), Spain
[3] Faculty of Medicine, Universitat de Barcelona, Barcelona, Spain.

Obesity and type-2 diabetes are associated with cognitive dysfunction. Since the hypothalamus is implicated in energy balance control and memory disorders, we hypothesized that specific neurons in this brain region are at the interface of metabolism and cognition. Acute obesogenic diet administration in mice impaired recognition memory due to defective production of the neurosteroid-precursor pregnenolone in the hypothalamus. Genetic interference with pregnenolone synthesis by Star deletion in hypothalamic POMC, but not AgRP neurons, deteriorated recognition memory independently of metabolic disturbances. Our data suggested that pregnenolone's effects on cognitive function were mediated via an autocrine mechanism on POMC neurons, influencing hippocampal long-term potentiation. The relevance of central pregnenolone on cognition was also confirmed in metabolically-unhealthy obese patients. Our data reveals an unsuspected role for POMC neuron-derived neurosteroids in cognition. These results provide the basis for a framework to investigate new facets of POMC neuron biology with implications for cognitive disorders.

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**NOTES**

## O.30. NEUROPHYSIOLOGICAL CHARACTERIZATION OF A MOUSE MODEL OF CPT1C DEFICIENCY: FROM SYNAPSES TO BEHAVIOR

**Iborra-Lázaro G**[1], Sánchez-Rodriguez I[1], Djebari S[1], Sánchez-Fernández N[2], Radosevic M[3], Castellanos-Esparraguera A[2], Gratacòs-Batlle E[2,3], Dalmau J[3,4,5,6], Navarro-López JD[1]* Soto D[2,3]* Jiménez-Díaz L[1]*

[1] Neurophysiology & Behavioral Lab, CRIB, School of Medicine of Ciudad Real, University of Castilla-La Mancha, Ciudad Real, Spain

[2] Laboratory of Neurophysiology, Deparment of Biomedicine, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, Barcelona, Spain.

[3] August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

[4] Department of Neurology, University of Pennsylvania, Philadelphia, USA.

[5] Centro de Investigación Biomédica en Red Enfermedades Raras (CIBERER), Valencia, Spain. 6Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

*Corresponding authors

CPT1C (Carnitine Palmitoyltransferase 1C) is a neuron-specific enzyme widely distributed throughout the entire central nervous system (CNS) and highly expressed in discrete brain areas including the hypothalamus, hippocampus and amygdala. It is located at the endoplasmic reticulum, where it regulates ceramide metabolism, triacylglicerol synthesis and, more recently, it has also been demonstrated its involvement in dendritic spine maturation and AMPA receptor synthesis and trafficking. Consistent with its widespread distribution in the CNS and its molecular functions, CPT1C plays a crucial role in hypothalamic control of food intake, energy homeostasis, motor function and hippocampal-dependent spatial memory. However, CPT1C might have additional functions that remain unexplored. Here, we carried out a systematic characterization of the role of CPT1C at different levels of complexity -molecular, synapses, neural networks and behavior-. First, CPT1C expression pattern in the CNS was studied by immunohistochemistry. Then, we assessed its physiological role in CPT1C knockout animals by evaluating locomotor activity, energy state and mood. Additionally, we investigated CPT1C involvement in motor learning, hippocampal-dependent spatial and habituation memory, and instrumental learning. Finally, to correlate neural activity with hippocampal-dependent memory processes, synaptic plasticity was evaluated ex vivo and electrocorticographic recordings were obtained in vivo. Our data confirmed the presence of CPT1C across almost all brain regions, with strong expression in the hippocampus and amygdala. CPT1C-deficient animals exhibited energy deficits and impaired locomotor activity, but no changes related to the emotional state were found. These animals also showed deficits in motor and instrumental learning, as well as spatial and habituation memory, these latter effects being explained by the long-term plasticity impairments observed at the CA3-CA1 hippocampal synapse, inefficient dendritic spine maturation and aberrant cortical oscillatory activity. Together, our results confirm the role of CPT1C in energy homeostasis but also reveal that CPT1C is required for learning and memory processes taking place in brain areas underlying motor, associative, and non-associative learning.

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**NOTES**

## SESSION 3B

## DISEASE MECHANISMS AND THERAPIES

### O.31. NOVEL CAV2.1-MODULATORS TO TREAT HEMIPLEGIC MIGRAINE

**Izquierdo-Serra M**[1,2], Serra SA[1], Ward SE[3,4], Beswick PJ[3], van den Maagdenberg AM[5] Fernández-Fernández JM[1]

[1] Laboratori de Fisiologia Molecular, Departament de Medicina i Ciències de la Vida, Universitat Pompeu Fabra, Barcelona.

[2] Laboratori de Neurofisiologia, Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona

[3] Sussex Drug Discovery Centre, School of Life Sciences, University of Sussex, Brighton, United Kingdom

[4] Medicines Discovery Institute, Cardiff University, Wales, United Kingdom

[5] Department of Neurology and Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Human mutations in the CACNA1A gene, encoding the pore-forming α1A subunit of the voltage-gated CaV2.1 calcium channel, cause most of the familial and sporadic hemiplegic migraine (FHM/SHM) cases. Hemiplegic Migraine (HM) mutations induce a gain-of-function in CaV2.1 channel that specifically enhances cortical excitatory transmission to favor initiation and propagation of cortical spreading depression, a key process in migraine pathophysiology. Accordingly, pharmacological evidences suggest that reduction of CaV2.1 activity can provide new therapeutic approach for HM and common migraine. Currently the only truly CaV2.1-selective inhibitors are peptide toxins, which are not suitable therapeutic tools, they have limited utility for in vivo studies. Therefore, low-molecular-weight compounds that selectively modulate CaV2.1 activity could emerge as new drugs to treat migraine. We have employed a high-throughput study using automated patch-clamp to identify novel selective CaV2.1-inhibitors. Starting from 80 compounds recognized as potential CaV2.1-blockers in silico, our results highlighted six novel low-molecular-weight compounds with higher selectivity for CaV2.1 inhibition (over other CaV channels). Next, in a low-throughput evaluation, we studied their effect on the wild-type “healthy” and gain-of-function FHM mutant CaV2.1 channels. We identified novel potent and selective inhibitors of CaV2.1 capable of preventing the excessive activity of CaV2.1 channels produced by human HM mutations, with minor effects on the wild-type channel (in order to reduce/prevent potential side effects). Furthermore, we tested if the reduction of excessive CaV2.1 activity was sufficient to prevent hyper-excitability on cortical networks observed in HM mouse model, a gain-of-function CaV2.1 knock-in animal. Interestingly, our study featured a selective CaV2.1 inhibitor that diminished excitability of the aberrant neuronal network with minor effects on the healthy cortical neuronal network from wild-type mice. This novel molecule that modulates the CaV2.1 channel activity and reverts its pathological gain-of-function effect may contribute to the development of a new, effective and safe treatment for both hemiplegic and common migraine.

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**NOTES**

## O.32. GLYCOGEN SYNTHASE KINASE-3 INHIBITION AFFECTS DOPAMINE METABOLISM BY DECREASINGTYROSINE HYDROXYLASE ACTIVITY

**Hamdon S**[1,2], Omar MY[1,2], Gil C[1,2] *, Ortiz J[1,2,3] *

[1] Institut de Neurociències, School of Medicine, Universitat Autònoma de Barcelona.
[2] Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona.
[3] Centro Investigación Biomédica en Red de Salud Mental, CIBERSAM, and Translational Neuroscience Unit, Parc Taulí University Hospital and Universitat Autònoma de Barcelona
* Equal contribution

The use of antipsychotic medications imposes a chronic struggle between the benefit of reducing psychotic symptoms [1, 2] and the possibilty of adverse effects [3, 4]. The activation of presynaptic D2 autoreceptors in the striatum inhibits dopamine synthesis [5]. This effect has been associated to changes in phosphorylation of tyrosine hydroxylase (TH) -the rate-limiting enzyme of brain dopamine biosynthesis-but the signaling mechanisms involved are not yet fully understood. We are currently examining the possibility that both the G-protein and β-arrestin signal transduction pathways could be involved in explaining these mechanisms. We hypothesize that glycogen synthase kinase-3 (GSK-3) plays a key role in the presynaptic D2 autoreceptor signal transduction pathway, which could be of interest as an alternative target for schizophrenia treatment. We tested the effect of GSK-3 inhibitors CHIR-99021 and SB-216763 on dopamine synthesis and accumulation in rat striatum slices ex vivo. We also compared their effects with lithium chloride (LiCl), which is known to have antipsychotic properties and inhibit GSK-3, as well as the β-arrestin biased D2 receptor agonist UNC9994 and the D2 full agonist quinpirole. HPLC-EC was used to assess dopamine accumulation, while dopamine synthesis was measured using HPLC-UV. Interaction between 2 factors was assessed by two-way analysis of variance (ANOVA) followed by Bonferroni test for post-hoc comparisons. One-way ANOVA was applied with Dunnett´s post-hoc test for comparison against control or Bonferroni post-hoc test for direct comparison between tested groups. Statistical analysis (treated with drug and respective control) was performed by the unpaired Student´s t-test. We have found that CHIR, SB, LiCl and UNC were all able to significantly decrease dopamine accumulation in rat striatum slices ex vivo. CHIR did so in a similar manner to UNC, while SB and LiCl had smaller but nonetheless significant effects.Given that DOPAC/DA ratio is a measure of DA turnover, our results showed that while DOPAC/DA remains constant for quinpirole and lithium, it significantly increases for UNC and CHIR. Further testing on CHIR revealed that it also significantly decreases dopamine synthesis. Given that L-DOPA is an indicator of TH activity, we also found a significant decrease in L-DOPA levels after CHIR treatment. And finally, comparing L- DOPA levels after CHIR treatment between striatal slices and homogenates revealed a significant decrease only with slices. Our results show that GSK-3 plays an important role in the dopamine synthesis pathway, by affecting the activity of TH. The fact that we only saw this in slices, not homogenates, discards direct interaction between CHIR and TH. The similarity that we saw in CHIR and UNC profiles suggest that they share some sort of mechanism of action. Since GSK-3 is part of the β-arrestin pathway, controlling dopamine hyperactivity through selective targeting could control psychotic symptoms without the adverse effects associated with conventional antipsychotics.

**NOTES**

## 0.33. GPR37 N-TERMINAL DOMAIN PROCESSING DEFINES AUTAPTIC RECEPTOR SIGNALLING

**Argerich J**[1,2], Fernández-Dueñas V[1,2], Ciruela F[1,2]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, Schoolof Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, 08907 L'Hospitalet de Llobregat, Spain
[2] Neuropharmacology & Pain Group, Neuroscience Program, Bellvitge Institute forBiomedical Research, 08907 L'Hospitalet de Llobregat, Spain.

GPR37 is an orphan G protein-coupled receptor (GPCR) which has gained attention dueto its implication in the pathogenesis of Parkinson's disease (PD). Interestingly, the N- terminus of the receptor (i.e., ecto-GPR37) is subject to metalloproteinase-mediated proteolysis, which leads to several receptor forms at the cell surface. In addition, PD patients have increased amounts of ecto-GPR37 in the cerebrospinal fluid, thus showingan increased expression of GPR37 cleaved isoforms in the brain. Here, we aimed at investigating the impact of ecto-GPR37 on receptor's function and cell viability. To thisend, we generated five GPR37 N-terminally truncated constructs ($GPR37^{\Delta1-54}$, $GPR37^{\Delta1-171}$, $GPR37^{\Delta1-199}$, $GPR37^{\Delta1-219}$, $GPR37^{\Delta1-247}$) based on the receptor isoforms found in human PD brain samples. Subsequently, we assessed their coupling to different transducers, namely GRK-2, β-Arrestin 2 and G proteins, in HEK293T cells. Thus, GPR37 full length, $GPR37^{\Delta1-54}$, $GPR37^{\Delta1-171}$ and $GPR37^{\Delta1-199}$ showed a robust coupling to GRK-2 and β-Arrestin 2. Also, these truncated forms activate serum response element(SRE)-related signalling pathways which are downstream to β-Arrestin 2. Interestingly, this signalling cascade is attenuated when GPR37 is truncated beyond aminoacid 219. Inaddition, the impact of GPR37 N-terminally truncated forms expression in SH-SY5Y cells viability was assessed upon 6-hydroxydopamine (6-OHDA) challenge. Thus, the neurotoxic effects of 6-OHDA in SH-SY5Y cells is modulated by the GPR37 N-terminal truncation. Overall, these results provide evidence that ecto-GPR37 may play a key role controlling receptor's constitutive activity and regulating cell viability, which may be relevant to understand its relationship with PD pathogenesis.

**NOTES**

## O.34. ROLE OF SAC1 IN NEURONAL MITOCHONDRIAL FISSION REGULATION

**Molins A**[1], Fadó R[1], Casals N[1,2]

[1] Departament de Ciències Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Internacionalde Catalunya, Sant Cugat del Vallès
[2] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición, Instituto deSalud Carlos III, Madrid

Mitochondrial fusion and fission are biological processes that regulate the shape, size and number of mitochondria within the cell, thus regulating their function. Alterations in such processes have been associated with multiple neurological disorders, including Alzheimer's and Parkinson's disease. However, due to the high complexity of mitochondrial dynamics, the molecular machinery and regulation pathways involved are still poorly understood. A recent study suggests the participation of phosphatydilinositol-4-phosphate (PI4P) in the activation of mitochondrial fission. PI4P is a lipid implicated in membrane-remodelling processes, synthesized by a phosphatidylinositol-4-kinase and dephosphorylated by the lipid phosphatase SAC1. SAC1 is a trans-membrane protein that shuttles between the endoplasmatic reticulum (ER) and the Golgi and dephosphorylates PI4P pools at the contactsites of these organelles, thereby regulating vesicle trafficking. Based on these previous studies, we hypothesized that SAC1 plays a role in the regulation of mitochondrial fission. To test this hypothesis, weused the neuronal cell line GT1-7 as a model. Our results show that SAC1 overexpression activates mitochondrial fission, whereas its silencing decreases it. Interestingly, we discovered that SAC1 is also located in the region of contact between the ER and mitochondria, where mitochondrial fission is initiated. Finally, we confirmed that SAC1 decreases PI4P levels in the trans-Golgi network, as expected. Therefore, this phosphatase appears to have a role in mitochondrial fission, although further experiments conducted in primary neuronal cultures are required to clarify whether SAC1 exerts its effect in ER-mitochondrial contact sites or by regulating other PI4P intracellular pools. Elucidating the mechanisms that underlie mitochondrial fission would not only improve the knowledge on this field but also the study of new therapeutic targets for the treatment of numerous neurological and metabolic diseases.

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**NOTES**

## O.35. TREATMENT OF MITOCHONDRIAL DISORDERS: METABOLIC INTERVENTION FOR THE TREATMENT OF COMPLEX I DEFICIENCIES BASED ON THEIR MOLECULAR PROFILES

**García-Adán B**, Musokhranova U, Grau C, Serradell A, O'Callaghan M, Rivera N, Oyarzábal A, García-Cazorla A

Synaptic Metabolism Lab. Department of Neurology. Institut de Recerca Sant Joan de Déu, CIBERER and metabERN. Barcelona, Spain

Mitochondrial complex I dysfunction is the most common cause of mitochondrial disorders. It is associated with a spectrum of progressive neuromotor manifestations, including encephalopathy, epilepsy, ataxia, and dystonia. Consequently, the marked clinical and genetic heterogeneity hinders the diagnosis and treatment of patients. In such cases, precision medicine is the best alternative to resolve the complexity of mitochondrial diseases and propose suitable therapeutical options. We report the personalized study of two unrelated individuals with mitochondrial complex I deficiency. Both patientscarried pathogenic variants in a complex I-related gene: *NDUFS1* and *NDUFAF6*, respectively. *NDUFS1* encodes a core subunit of complex I, whereas *NDUFAF6* codes for a protein involved in the early assembly stages of the same complex.

To define the mitochondrial affectation in each case, we first investigated some aspects of its function in fibroblasts derived from each patient. We identified a decrease in total levels of several mitochondrial respiratory chain complexes, including complex I. Besides, we found alterations in cellular bioenergetics and redox homeostasis. Furthermore, we identified abnormalities in the mitochondrial network structure, including altered morphology and expression levels of proteins involved in mitochondrial dynamics. Under this scenario, we tested *in vitro* the potential treatment with a nutraceutical compound known to enhance mitochondrial biogenesis. Noteworthy, we corrected several alterations such as ATP deficiency without further increasing oxidative stress. Based on the *in vitro* results, both patients have been treated with the studied compounds, resulting in a substantial improvement in different clinical outcomes.

Our results highlight the potential of personalised therapy, starting in an accurate molecular study up to an individually-tailored treatment. This approach to neuropediatric disease has been extended by our group to other neurometabolic disorders, with similar positive results.

**NOTES**

## O.36. HUMAN BRAIN AGING INDUCES CHANGES REGION-SPECIFIC IN LIPID PROFILE

**Mota-Martorell N**[1], Andrés-Benito P[2], Martín-Gari M[1], Sol J[1], Obis E[1], Fernández-Bernal A[1], Portero-Otín M[1], Ferrer I [2,3], Jové M[1], Pamplona R[1]

[1] Department of Experimental Medicine, University of Lleida-Lleida Biomedical Research Institute (UdL-IRBLleida), 25198, Lleida, Spain.
[2] Center for Biomedical Research On Neurodegenerative Diseases (CIBERNED), Institute of Health Carlos III, 28220, Madrid, Spain.
[3] Department of Pathology and Experimental Therapeutics, University of Barcelona, L'Hospitalet de Llobregat, 08907, Barcelona, Spain.

Fatty acids are key components in the structural diversity of lipids and play a strategic role in their functional properties which determine the integrity of neuronal and glial cell membranes, the generation of lipid signaling mediators, and the chemical reactivity of acyl chains. The present study analyzes using gas chromatography the fatty acid profiles of 13 regions of the human central nervous system in healthy individuals ranging from 40 to 80 years old. The outcomes suggest the existence of general traits in fatty acid composition such as an average chain length of 18 carbon atoms, high monounsaturated fatty acid content, and predominance in polyunsaturated fatty acids of those of series n-6 over series n-3 which are shared by all brain regions regardless of age. Our results also show a general sustained and relatively well-preserved lipid profile throughout the adult lifespan in most studied regions (olive, upper vermis, substantia nigra, thalamus, hippocampus, putamen, caudate, occipital cortex, parietal cortex, entorhinal cortex, and frontal cortex) with minor changes that are region-dependent. In contrast, of particular relevance is the involvement of the inferior temporal cortex and cingulate cortex. It is proposed that during normal human brain aging, the lipid profile is resistant to changes with age in most human brain regions to ensure cell survival and function, but some particular regions involved in specific memory domains are greatly affected.

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**NOTES**

## SESSION 4A

## MENTAL AND BEHAVIORAL DISORDERS

### O.37. PACAP-PAC1R MODULATES FEAR EXTINCTION VIA THE VENTROMEDIAL HYPOTHALAMUS

Velasco ER[1], Florido A[1,2], Flores Á[1,3], Senabre E[4], Gomez-Gomez A[5], Torres A [6,7,8], Roca A[6], Norrholm S[9], Newman EL[10], Das P[11], Ross RA[11], Lori A[12], Pozo OJ[5], Ressler KJ[10], Garcia-Esteve LL[6,7,8], Jovanovic T[9], **Andero R**[2,13,14,15]

[1] Institut de Neurociències, Universitat Autònoma de Barcelona, , Bellaterra, Spain.
[2] Departament de Psicobiologia i de Metodologia de les Ciències de la Salut, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain.
[3] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Barcelona, Spain
[4] Laboratory of Neuropharmacology-NeuroPhar, Department of Experimental and Health Sciences, University Pompeu Fabra, Barcelona, Spain.
[5] Integrative Pharmacology and Systems Neuroscience Research Group, Neurosciences Research Program, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain.
[6] Perinatal Mental health Unit, Department of Psychiatry and Clinical Psychology, Institute of Neuroscience, Hospital Clínic, IDIBAPS, Barcelona, Spain.
[7] Vulnerability, Psychopathology and Gender research group, Generalitat Catalunya, Barcelona, Spain.
[8] Programme for the Prevention and Treatment of Psychic Effects in Sexually Assaulted Women. Hospital Clínic de Barcelona. Spain.
[9] Department of Psychiatry and Behavioral Neuroscience, Wayne State University, Detroit, USA.
[10] McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont, MA, USA.
[11] Department of Neuroscience, Albert Einstein College of Medicine, Psychiatry Research Institute of Montefiore and Einstein, New York, USA.
[12] Department of Psychiatry & Behavioral Sciences, Emory University, Atlanta, GA, USA.
[13] Centro de Investigación Biomédica En Red en Salud Mental (CIBERSAM), ISC III, Madrid, Spain.
[14] Unitat de Neurociència Traslacional, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT), Institut de Neurociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. [15] ICREA, Barcelona, Spain.

Exposure to traumatic stress can lead to fear dysregulation, which has been associated with posttraumatic stress disorder (PTSD). The incidence and severity of PTSD are higher in women than men secondary to environmental and biological factors. Previous work showed that a polymorphism in the PACAP-PAC1R (pituitary adenylate cyclase-activating polypeptide) system is associated with PTSD risk in women, and PACAP (ADCYAP1) - PAC1R (ADCYAP1R1) are highly expressed in the hypothalamus. Here, we show that female mice subjected to acute stress immobilization (IMO) have fear extinction impairments related to Adcyap1 and Adcyap1r1 mRNA upregulation in the hypothalamus, PACAP/c-Fos downregulation in the medial amygdala (MeA), and PACAP/FosB/ΔFosB upregulation in the ventromedial hypothalamus dorsomedial part (VMHdm). DREADD-mediated inhibition of MeA neurons projecting to the VMHdm during IMO rescues both PACAP upregulation in VMHdm and the fear extinction impairment. We also found that women with the risk genotype of ADCYAP1R1 rs2267735 SNP show impaired fear extinction.

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**NOTES**

## O.38. ESTROGENS MODULATE FOOD ADDICTION SIGNATURES IN FEMALES

**Casadó-Anguera V**[1] , Dwaib HS[2,3,4], Quesada-López T[5,6], Domingo-Rodriguez L[1], Florido A[7,8], Andero R[7,8,9,10,11], Giralt M[5,6], Villarroya F[5,6], Martín-García E [1]*, Maldonado R[1]*

[1] Department of Medicine and Life Sciences, Universitat Pompeu Fabra UPF, Barcelona.

[2] Department of Pharmacology and Toxicology,The American University of Beirut, Beirut, Lebanon

[3] Department of Nutrition and Food Sciences Faculty of Agriculture and Food Sciences The American University of Beirut, Beirut, Lebanon

[4] Department of Clinical Nutrition, Palestine Ahliya University, Bethlehem, Palestine.

[5] Department of Biochemistry and Molecular Biomedicine, Faculty of Biology and Institute of Biomedicine (IBUB), Universitat de Barcelona, Barcelona.

[6] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Barcelona.

[7] Institut de Neurociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

[8] Departament de Psicobiologia i de Metodologia de les Ciències de la Salut, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona.

[9] Centro de Investigación Biomédica En Red en Salud Mental (CIBERSAM), ISC III, Madrid.

[10] Unitat de Neurociència Traslacional, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT), Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona.

[11] ICREA, Barcelona.

Food addiction is characterized by the loss of behavioral control and compulsive food intake. Its prevalence is 5-15%, being more frequent in women than in men. During menopause, anxiety and fatigue have been associated with craving for refined carbohydrates and sugar, increasing the vulnerability of these women to food addiction. In this study we aimed to elucidate the effect of estrus cycle hormones in the development of food addiction. Therefore, we applied a food addiction mouse model in sham and ovariectomized (OVX) females. The percentage of sham females who developed a food addiction-like behavior was 35%, higher than that reported in previous similar studies for males (19-26%). Importantly, a higher percentage of OVX mice (50%) accomplished the criteria of food addiction, suggesting a protective effect of estrogens. Furthermore, addicted mice showed differential gene expression that was probably ovariectomy-dependent. Indeed, there was downregulation of Drd2 and Adora2a in the dorsal striatum of addicted sham females and in the nucleus accumbens of addicted OVX mice. Furthermore, in inguinal white adipose tissue (iWAT), addicted sham females showed decreased expression of genes associated to thermogenic activity such as Ucp-1, Th, Fgf21 and Ppargc1 and addicted OVX females presented a downregulation of genes encoding glucose transporter 1 (Slc2a1) and 4 (Slc2a4) in brown adipose tissue and iWAT, respectively. Thus, our results could facilitate the understanding of the neurobiological basis underlying food addiction disorder in females, highlighting the relevance of considering sex and stage of life differences when designing new treatment strategies.

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**NOTES**

## O.39. PROGRESSIVE BEHAVIOURAL AND PROTEOMIC CHANGES ASSOCIATED WITH STRESS AND POTENTIAL NON-INVASIVE THERAPEUTIC STRATEGIES

**Sancho-Balsells A**[1,2,3,4], Rellano-Ginés A[5], Xifró X[6] , Alberch J[1,2,3,4] , Giralt A[1,2,3,4]

[1] Universitat de Barcelona. Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)
[4] Institut de Neurociències, Universitat de Barcelona. Barcelona, Spain
[5] REGEnLIFE, Montpellier, France
[6] Universitat de Girona. Girona, Spain

Major depression (MD) is a common, relapsing mental illness that affects millions of people worldwide. One of the most studied risk factors associated with MD is chronic stress. Current treatment is ineffective in 30% of patients so there is a need to fully understand the pathophysiology and find new non-invasive therapeutic approaches. Photobiostimulation (PBS) has raised as a potential therapeutic strategy to ameliorate depressive symptoms.

Here, we want to evaluate if the changes induced by stress are accumulative and time dependent. To do so, we divide our mice into control non-stressed (NS), short-term stress mice (STS) and long-term stress mice (LTS). STS mice received only two days of stress whereas LTS mice underwent the chronic unpredictable mild stress protocol (CUMS) for 28 days. After the stress protocol, we analyse the biochemical, behavioural, and microbial changes induced by stress. Additionally, we want to test if these alterations can be modulated using a non-invasive method such as PBS.

Results indicate that LTS induces much more severe depressive-like symptoms than STS as demonstrated in the body weight, the fur appearance, and in the anxiety levels in the open field. Moreover, biochemical analysis reveals huge differences depending on the duration of the stress. Furthermore, PBM ameliorates some of the cognitive deficits induced by chronic stress and rescues CA1 hippocampal spine loss.

In summary, our work suggests that there is a progression in the biochemical, behavioural and microbial changes induced by stress. Our results also indicate that PBM can improve some of the sequalae induced by chronic stress.

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**NOTES**

## O.40. VALIDATION OF APP/PS1 MICE AS A PRECLINICAL MODEL OF PSYCHOSIS IN ALZHEIMER'S DISEASE

**Gómez-Acero L**[1,2], Sánchez-Fernández N[1,2], Fernández-Dueñas V[1,2], Ciruela F[1,2], Aso E[1,2]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Spain.
[2] Neuropharmacology & Pain Group, Neuroscience Program, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Spain.

Psychosis is present in 40-60% of patients with Alzheimer's disease (AD) and is associated with a worse prognosis and a more severe cognitive impairment. Psychotic symptoms, such as hallucinations and delusions, usually appear as illness severity and duration increase. However, current antipsychotics have poor efficacy and important side effects in such demented patients. These limitations raise the necessity of developing preclinical models of psychosis in AD to screen potential treatments. In this work, we aimed to validate whether double transgenic APP/PS1 mice recreate the psychotic symptoms observed in AD patients. To this end, we evaluated 4 and 12-month-aged APP/PS1 and WT mice, reflecting an early and a late stage of AD, respectively. Then, we evaluated the psychosis-associated symptoms at different behavioral paradigms. First, APP/PS1 mice were exposed to the Prepulse Inhibition (PPI) test and showed a decrease of the sensorimotor gating at 12 months. Moreover, a decrease in sociability index and a diminished social memory were found exclusively in the 12-month aged APP/PS1 mice in the three chambers test. Additionally, increased anxiety levels were evidenced in 12 month-aged APP/PS1 mice compared to wild-type littermates in the elevated plus maze. In contrast, no differences were observed between genotypes in the early stage of 4-month-age in none of the three behavioural paradigms. Current understanding points towards a dysregulation of the dopaminergic, adenosinergic and endocannabinoid systems, among others, as relevant substrates for the development of psychotic disorders. Consequently, we evaluated some key components of such systems in the striatum of the transgenic and WT mice. Thus, we observed altered levels of DAT, ENT1, A2AR and CB1R. Collectively, these results suggest that 12 month-aged APP/PS1 mice may constitute a valid preclinical model for psychosis in AD and that the adenosinergic, dopaminergic and cannabinoid system may be implicated in this phenotype.

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**NOTES**

## O.41. miR-135 AS A NEW TARGET FOR ANTIDEPRESSANT THERAPY: PRECLINICAL STUDY

**Pavia-Collado M**[1,2,3,4], Manashirov S[3,4,5], Ruiz-Bronchal E[1,2], Rodríguez-Navarro I[1,2], Campa L[1,2,3], Bortolozzi A[1,2,3]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), Consejo Superior de Investigaciones Científicas (CSIC), Barcelona
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona
[3] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid
[4] miCure Therapeutics LTD., Tel-Aviv, Israel
[5] Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, Germany

Major depressive disorder (MDD) is a major health problem worldwide. Most prescribed antidepressants show limited efficacy and delayed onset of action. Accumulating data support the association between MDD pathophysiology and changes in miRNA pathways. Indeed, low miR-135 levels were found in blood and postmortem brain tissue from depressed patients. In vitro and in vivo studies showed that miR-135 regulates the expression of components of serotonin neurotransmission, including serotonin transporter (SERT) and 5-HT1A receptor (5-HT1AR) transcripts. The current study aimed to investigate the antidepressant-like effects of a synthetic miR-135 in a mouse model.

We have designed a sertraline-conjugated synthetic miR-135 oligonucleotide (c-miR-135) to be accumulated in serotonin (5-HT) neurons after local infusion into dorsal raphe nucleus (DRN) or administered intranasally in mice. Histological and biochemical approaches and stress-related behavioral tests were performed to examine the potential antidepressant-like effects of c-miR-135. Intranasal c-miR-135 administration resulted in its selective accumulation in raphe 5-HT neurons. This elicited a marked antidepressant-like effect in the tail suspension test but did not affect anxiety-like behaviors in the dark-light box. In parallel, a single dose of c-miR-135 (2.5 mg/40 ul/day, intranasal) significantly decreased SERT and 5-HT1AR protein levels in the DRN, without inducing the loss of TPH-positive neurons, astrogliosis, or microgliosis. The changes on SERT and 5-HT1AR protein levels were found up to 10 days after treatment with c-miR-135. Moreover, intranasal c-miR-135 administration modified the effects of 5-HT1AR agonist 8-OH-DPAT and SERT inhibitor citalopram on extracellular 5-HT levels in the medial prefrontal cortex of mice. Interestingly, a single dose of c-miR-135 reversed depressive-like behaviors in corticosterone-treated mice.

These results suggest that miRNAs are useful as molecular targets to develop new therapeutic strategies for MDD. C-miR-135 evokes antidepressant-like responses by selectively targeting neuronal populations, opening a way for translational studies.

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**NOTES**

## O.42. A ZEBRAFISH MODEL OF NEUROTOXICITY BY BINGE-LIKE METHAMPHETAMINE EXPOSURE

**Bedrossiantz J**[1], Bellot M[2], Dominguez-García P[2], Faria M[1], Prats E[3], Gómez-Canela C[2], López-Arnau R[4], Escubedo E[4], Raldúa D[1]

[1] Institute for Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain,
[2] Department of Analytical and Applied Chemistry (Chromatography Section), School of Engineering, Institut Químic de Sarrià-Universitat Ramon Llull, Barcelona, Spain,
[3] Research and Development Center (CID-CSIC), Barcelona, Spain,
[4] Department of Pharmacology, Toxicology and Therapeutic Chemistry, Pharmacology Section and Institute of Biomedicine (IBUB), Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

Hyperthermia is a common confounding factor for assessing the neurotoxic effects of methamphetamine (METH) in mammalian models. The development of new models of methamphetamine neurotoxicity using vertebrate poikilothermic animals should allow to overcome this problem. The aim of the present study was to develop a zebrafish model of neurotoxicity by binge-like methamphetamine exposure. After an initial testing at 20 and 40 mg/L for 48 h, the later METH concentration was selected for developing the model and the effects on the brain monoaminergic profile, locomotor, anxiety-like and social behaviors as well as on the expression of key genes of the catecholaminergic system were determined. A concentration- and time-dependent decrease in the brain levels of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) was found in METH exposed fish. A significant hyperactivity was found during the first hour of exposure, followed 3 h after by a positive geotaxis and negative scototaxis in the novel tank and in the light/dark paradigm, respectively. Moreover, the behavioral phenotype in the treated fish was consistent with social isolation. At transcriptional level, th1 and slc18a2 (vmat2) exhibited a significant increase after 3 h of exposure, whereas the expression of gfap, a marker of astroglial response to neuronal injury, was strongly increased after 48 h exposure. However, no evidences of oxidative stress were found in the brain of the treated fish. Altogether, this study demonstrates the suitability of the adult zebrafish as a model of METH-induced neurotoxicity and provides more information about the biochemical and behavioral consequences of METH abuse.

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**NOTES**

## 0.43. DISENTANGLING THE IMPACT OF INHIBITORY HIPPOCAMPAL NEURONS IN ENGRAM FORMATION IN DOWN SYNDROME

**Sabariego-Navarro M**[1], Zamora-Moratalla A[1], Dierssen M[1,2,3,4]

[1] Center for Genomic Regulation, The Barcelona Institute for Science and Technology, Barcelona, Spain
[2] Neurosciences Research Program, Hospital del Mar Medical Research Institute, Barcelona, Spain
[3] University Pompeu Fabra, Barcelona, Spain
[4] Biomedical Research Networking Center for Rare Diseases (CIBERER), Barcelona, Spain

In the last years the concept of engram as a stable mnemonic footprint is gaining momentum. Engram cells can now be tagged and manipulated through chemo and optogenetic techniques, reactivating or suppressing a memory in an artificial way. However, less is known about the engram pathology. Our lab has shown that engram neuron allocation is impaired in Down syndrome, but the mechanisms are still not elucidated. Previous studies in the field support the idea that alterations in the excitation-inhibition balance contribute to Down syndrome cognitive impairment, with evidences indicating an overinhibition in cortical and hippocampal circuits. Our hypothesis is that hippocampal engram formation is disrupted in Down syndrome due to an alteration in inhibitory microcircuits. Our work has shown that the different inhibitory interneurons subtypes are unevenly affected in the hippocampal formation. Breeding trisomic mice with transgenic mice expressing the Cre recombinase under the promoter of Parvalbumin and Somatostatin, the main inhibitory interneurons subtypes, and using viral constructions containing chemo and optogenetic tools, we modulate the activity of inhibitory interneurons during the formation of an engram using a contextual fear-conditioning paradigm, analyzing the impact of this modulation in posterior engrams reactivation. We also perform in vitro electrophysiology in order to disentangle the specific properties of these interneurons subtypes during their activation, and also analyze their morphological properties and further interactions with engram cells.

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**NOTES**

## SESSION 4B

## SENSORY AND MOTOR SYSTEMS

### O.44. LOSS OF TRESK BACKGROUND POTASSIUM CHANNEL ENHANCES ACUTE AND CHRONIC ITCH

Andres-Bilbe A[1,2], Castellanos A[1,2], Pujol A[1], Llimós J[1,2], Comes N[1,2], Gasull X[1,2], **Callejo G**[1,2]

[1] Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain

[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

TRESK (K2P18.1) is a background K+ channel expressed in sensory neurons, where it modulates the resting membrane potential, action potential firing and neuronal excitability. A subset of these sensory neurons, which express specific TRPs and Mas-related G protein-coupled receptors (Mrgprs), are activated by pruritogens and mediate itch sensation. Because TRESK is involved in somatosensitivity and pain perception, we evaluated the contribution of this channel to pruritic sensitivity and its potential as a target for the treatment of chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma and different types of dermatitis. By combining, RNA in situ hybridization, calcium imaging, electrophysiological and behavioral approaches, we found that TRESK is involved in the modulation of non-histaminergic itch. TRESK colocalizes in MrgprD+ and MrgprA3+ sensory neurons. Different populations of primary cultured sensory neurons from both wild-type and TRESK knockout mice were activated by chloroquine (CQ), β-alanine, BAM8-22 or histamine in calcium imaging experiments. At the behavioral level, subcutaneous injection of chloroquine in the cheek model produced an acute scratching response, which was significantly enhanced in mice lacking TRESK. Interestingly, TRESK KO mice also showed alterations in mice models of chronic itch. Induction of Allergic Contact Dermatitis or Dry Skin showed a significantly higher scratching response in mice lacking TRESK compared to their wild-type counterparts. In the mouse model of imiquimod-induced psoriatic itch, the absence of TRESK produced a significantly enhanced scratching behavior, which developed earlier and was more robust. In summary, our data indicate that TRESK is involved in regulating the excitability of a subset of sensory neurons that mediate histaminergic-independent itch. Given the prominent role of this neuronal subpopulation in chronic itch diseases, TRESK appears as a new potential candidate for therapeutic intervention.

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**NOTES**

## O.45. THE INTERACTION BETWEEN CARBON MONOXIDE AND HYDROGEN SULFIDE DURING CHRONIC OSTEOARTHRITIS PAIN IN MICE

**Batallé G**, Bai X, Pol O

Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau, Hospital de la Santa Creu i Sant Pau and Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

A relationship between carbon monoxide (CO) and hydrogen sulfide ($H_2S$) has been described in different pathological conditions, but their interaction in the modulation of osteoarthritis pain has not yet been investigated. In a female mouse model of osteoarthritis pain, we assessed the interaction between both gaseous neurotransmitters on the inhibition of the mechanical allodynia and grip strength deficits provoked by osteoarthritis by assessing: (1) the effects of systemic co-administration of a CO-releasing molecule, tricarbonyldichlororuthenium(II)dimer (CORM-2), or a heme oxygenase 1 (HO-1) inducer, cobalt protoporphyrin IX (CoPP), with two $H_2S$ donors, diallyl disulfide (DADS) and morpholin4-ium 4-methoxyphenyl(morpholino) phosphinodithioate dichloromethane complex (GYY4137); (2) the reversion of the antiallodynic effects and recovery of grip strength induced by $H_2S$ donors with specific Nrf2, HO-1, and NAD(P)H: quinone oxidoreductase 1 (NQO1) inhibitors; (3) the effects of treatment with DADS and GYY4137 on the expression of Nrf2 and antioxidant enzymes (HO-1, NQO1, superoxide dismutase 1, and glutathione S-transferase mu 1) in dorsal root ganglia and periaqueductal grey area. Results demonstrated that HO-1/CO or $H_2S$ activation inhibited the mechanical allodynia, but only the $H_2S$ donors decreased the loss of grip strength caused by osteoarthritis. The co-administration of CORM-2 or CoPP with DADS or GYY4137 potentiated the antiallodynic and recovery of grip strength effects of each of these compounds. Results also revealed the participation of the Nrf2 signaling pathway in the antinociceptive actions induced by DADS and GYY4137 at the pharmacological and biochemical levels. These data reveal a positive interaction between $H_2S$ and CO in modulating osteoarthritis pain.

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**NOTES**

## O.46. CPT1A SILENCING IN AgRP NEURONS IMPROVES PHYSICAL PERFORMANCE VIA SKELETAL MUSCLE REMODELING

Ibeas K[1], Reyes M[1], Gómez R[1], Palacios-Marin I[1], Romero M[1], Navarro D[1], Van Beurden S[1], Griñán-Ferré C[3], Pallás M[3], Castell M[1,2], Herrero L[1,2], Mera P[1,2*], **Serra D**[1,2*]

[1] Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, 08028 Barcelona, Spain
[2] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain
[3] Department of Pharmacology, Toxicology and Therapeutic Chemistry, School of Pharmacy and Food Sciences, Universitat de Barcelona, 08028 Barcelona, Spain

Introduction: Obesity is recognized as one of the major public health problems worldwide. Physical activity has emerged as one of the best therapies to fight against obesity. Exercise exhibits several benefits on health, including improvements in body weight, appetite, and glucose metabolism. Some factors are released into the circulation during exercise to maintain the whole-body homeostasis. Among these factors, leptin and insulin play a key role in activating the arcuate nucleus (ARC) of the hypothalamus. Specifically, orexigenic neurons expressing agouti-related protein (AgRP) are activated in response to dynamic variations in the metabolic state, including those occurring during exercise. In addition, fatty acid metabolism plays an important role in the brain-muscle crosstalk. Carnitine palmitoyltransferase 1a *(Cpt1a)* is a rate-limiting enzyme of mitochondrial fatty acid oxidation involved in the regulation of hypothalamic energy balance. However, the function of *Cpt1a* in AgRP neurons during exercise is poorly understood.
Objectives: To elucidate the potential role of *Cpt1a* in AgRP neurons and the identification of novel pathways involved in the response to physical activity.
Methods: We have generated a mutant mouse model lacking *Cpt1a* specifically in AgRP neurons (*Cpt1a* AgRP$^{-/-}$). To evaluate the physical capacity of *Cpt1a* AgRP$^{-/-}$ mice, we have performed different exercise tests where we analyzed the mice's behavior, strength, and physical activity. Moreover, we have done a histological analysis of the skeletal muscle and myofiber types using immunofluorescence staining and the gene expression of different myosin heavy chain isoforms in the gastrocnemius (GAS) and tibialis anterior (TA) muscles.
Results: Our results suggest that specific deletion of *Cpt1a* in AgRP neurons improves endurance, locomotion and motor coordination compared to control mice. Nonetheless, no changes in anxiety-related behavior and muscle strength were observed. In addition, *Cpt1a* AgRP$^{-/-}$ mice show a reduction in the GAS and TA muscle mass. The cross-section area (CSA) of these muscles is smaller compared to control mice. This low CSA is associated with an increase in type I myofibers exhibiting a fiber remodeling from type II (glycolytic) to type I (oxidative) myofibers.
Conclusion: *Cpt1a* in AgRP neurons is necessary to modulate physical activity and myofiber remodeling in the skeletal muscle. Future studies would clarify the role of *Cpt1a* as a potential therapy for enhancing locomotor skills and muscle function that are damaged in some pathologies such as obesity.

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**NOTES**

## O.47. CANNABIDIOL AMELIORATES MITOCHONDRIAL DISEASE VIA THE ACTIVATION OF PPARγ

**Puighermanal E**, Menardy F, Gella A, Sanz E, Quintana A

Institut de Neurociències, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Bellaterra (Spain).

Mitochondria are essential organelles that generate the majority of cellular ATP. Mutations in either nuclear or mitochondrial genomes may cause mitochondrial dysfunction and lead to a heterogeneous group of disorders known as primary mitochondrial diseases (MD), for which no treatments are approved. MDs are usually progressive, and often cause significant disability and premature death. Among them, Leigh syndrome (LS) is the most common form of pediatric MD and is generally characterized by a neuromuscular affectation.

Here, we report that daily CBD administration significantly extends lifespan and improves clinical complaints in two mouse models of LS. Noteworthy, we found that CBD delays motor decline and the appearance of neurogenerative signs, ameliorates social deficits, and decreases both the duration and intensity of thermally-induced seizures. These beneficial effects are correlated with decreased neuroinflammation in the globus pallidus, one of the main brain areas affected in LS. Moreover, we found that CBD's therapeutic effects require the activation of peroxisome protein activator receptor gamma (PPARγ), a nuclear receptor involved in mitochondrial function, energy metabolism and inflammatory responses. Altogether, our study unveils a PPARγ-dependent CBD beneficial effect on a devastating form of MD.

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**NOTES**

## O.48. MITOCHONDRIAL DYSFUNCTION IN RETT SYNDROME: STUDYING A NEUROLOGICAL DISORDER FROM SYNAPTIC METABOLISM PERSPECTIVE TO FIND NEW TREATMENT OPTIONS

**Musokhranova U**[1], Oyarzábal A[1], Pizcueta P[2], García-Cazorla A[1]

[1] Synaptic Metabolism Lab. Department of Neurology. Institut de Recerca Sant Joan de Déu, CIBERER and metabERN. Barcelona, Spain

[2] Minoryx Therapeutics, Department of Biology, Barcelona, Spain

Rett syndrome is a neurodevelopmental disease affecting 1:10,000 girls, usually due to *MECP2* mutations. It is characterized by a regression in the neuronal development, resulting in the loss of acquired capabilities and arousal of epileptic crisis. While it is a neurotransmission and neuronal maturation disorder, attention towards bioenergetics has been brought, and can be explored to find therapeutic options. We have focused our research on the analysis of mitochondrial homeostasis in Rett models and whether it can be modulated with therapeutic purpose. First, we analyzed mitochondrial performance in Rett patients' fibroblasts, for which we report a severemitochondrial dysfunction characterized by defective bioenergetics and altered mitochondrial dynamics andROS production. When we treated the fibroblasts with a PPARγ agonist, we registered a recovery in ATP production capacity and a decrease in ROS generation. Proved that mitochondrial homeostasis is altered and that it can be effectively modulated with a PPARγ agonist, we moved towards the analysis of mitochondrial function and targeting in animal models. Resembling the patients, MeCP2 female mice go through an asymptomaticphase to later develop the symptomatology. We observed mitochondrial dysfunction already in pre-symptomatic mice (altered dynamics and antioxidant protein expression), suggesting that the mitochondrial malfunction plays a role in the phenotype development and progression. Treatment of symptomatic mice with a PPARγ agonist resulted in behavioral improvement (explorative activity and motor coordination) through mitochondrial dysfunction amelioration (especially regarding ATP production in cerebellum). Neuronal maturation and activation markers were also altered and corrected by the treatment. Our results reaffirm mitochondria as an effective target for the treatment of an archetypical neurodevelopmental disorder, and endorse a clinical trialwith the mentioned PPARγ agonist. Moreover, we highlight the mitochondrial dysfunction even before the symptoms' onset, setting mitochondria as a highly relevant target to modify the natural history of Rett syndrome.

**NOTES**

## O.49. PKA-DEPENDENT SNAP-25 AND SYN-1 PHOSPHORYLATION ARE DIFFERENTLY REGULATED BY THE NEUROMUSCULAR ACTIVITY

**Polishchuk A**, Cilleros-Mañé V, Just-Borràs L, Durán M, Balanyà Segura M, Tomàs M, Garcia N, Tomàs J, Lanuza MA

Unitat d'Histologia i Neurobiologia (UHN), Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Spain.

At the neuromuscular junction (NMJ), PKA enhances ACh release maybe phosphorylating targets from the synaptic vesicle (SV) exocytotic cycle, although this is unknown. Synaptosomal associated protein (SNAP-25), which is part of the SNARE complex, and Synapsin-1 (Syn-1), which controls the release of the SV from the cytoskeleton to promote their docking, are PKA targets that highly influence the SV exocytosis. Although ACh release mechanism is regulated by presynaptic stimulus and retrogradely by the resulting muscle contraction, PKA regulation by the pre- and postsynaptic activities had not been studied until now. To separate the effect of presynaptic activity from that of the resulting muscle contraction on PKA subunits and its activity, the rat phrenic nerve was stimulated (1Hz, 30min) with and without contraction (abolished by μ-conotoxin GIIIB). PKA was pharmacologicaly inhibitited (H-89) to assess the interactions of PKA and its targets (SNAP-25 and Syn-1). We used Western blotting and cytosol/membrane translocation by subcellular fractionation.

We demonstrate that the pre- and postsynaptic activities differentially regulate the PKA subunit dynamics to be catalytic active at the NMJ and to phosphorylate SNAP-25 and Syn-1. Synaptic Cβ subunit regulated by RIIβ or RIIα subunits controls activity-dependent phosphorylation of SNAP-25 and Syn-1 respectively. Muscle contraction retrogradly downregulates presynaptic activity-induced pSyn-1 while that enhances pSNAP-25 T138. We hypothesize that both actions could coordinately contribute to decrease the neurotransmitter release at the NMJ.

These results provide a molecular mechanism of the bidirectional communication between nerve terminals and muscle cell to balance the optimal process of ACh release.

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**NOTES**

## O.50. CHARACTERIZATION OF THE AUTOPHAGY FLUX IN COMPLEX NEUROPEDIATRIC MOVEMENT DISORDERS TO DEFINE NEW PERSONALIZED THERAPIES

**Serradell A**, Darling A, Grau C, Oyarzabal A, García-Cazorla A

Synaptic Metabolism Lab. Department of Neurology. Institut de Recerca Sant Joan de Déu, CIBERER and metabERN. Barcelona, Spain

Autophagy is a conserved process of degradation through which the cell removes dysfunctional components. It is a complex and dynamic route with many agents implicated both in its progression and regulation. Mutations in genes affecting this pathway are known to be causative of many diseases.

In this work we have studied mutations in *WDR45*, that result in the neuropediatric movement disorder BPAN (β-propeller protein-associated neurodegeneration), characterized by an accumulation of brain ironand highly variable clinical phenotype including ataxia, dystonia and neurological impairment. Neither the exact role of *WDR45* nor the precise pathophysiology of BPAN are fully understood, resulting in the lack of therapeutic options for this devastating disease.

Complementing a deep clinical characterization, we have studied the autophagy flux progression inpatient fibroblasts. As it is a dynamic process, autophagy is hard to asses, and we have to infer the pathway progression through the analysis of several implicated agents. For that, we have treated both patient and control cells with in a set of conditions either inhibiting or activating autophagy at different stages of the flux, such as late-phase vacuolar vacuolar $H^+$ ATPase inhibitor (Bafilomycin A1), nucleationinhibitor 3-Methyladenine or culture with the autophagy-enhancer medium Earle's balanced salts solution (EBSS). We have evaluated autophagy flux progression through the analysis of several markers such as LC3BII/I ratio, LAMP1, Beclin or p62 expression and aggregation. Our results point towards a defective autophagy flux, with alterations in the early stages progression. Understanding and delimiting the pathophysiological basis of the disease are not only essential for the definition of therapeutic targets but also are allowing us to assess the response to different modulators in a personalized way.

**NOTES**

## POSTER SESSION

## GLIAL CELLS AND INFLAMMATION

### P.1. BORDER-ASSOCIATED MACROPHAGES INFLUENCE THE ENDOTHELIAL RESPONSE TO BRAIN ISCHEMIA

**Figuerola S**, Pedragosa J, Gallizioli M, Nova J, Petegnief V, Planas AM

Department of Neuroscience and Experimental Therapeutics, Institute for Biomedical Research of Barcelona (IIBB), Spanish National Research Council (CSIC), IDIBAPS, Barcelona, Spain.

Border-associated macrophages (BAMs) are brain macrophages that reside at the edges of the brain parenchyma in strategic locations for communication with the periphery. However, their role in brain pathology remains unknown. In this study we hypothesized that BAMs interact with the vasculature and affect the response of vascular endothelial cells (EC) after brain ischemia. In order to investigate the role of BAMs we took advantage of the fact that subsets of these macrophages express CD169 (Siglec1) under steady-state conditions. We obtained CD169-DTR mice, which express the diphtheria toxin receptor under the promoter of CD169. Administration of diphtheria toxin (DTx) i.p. three alternate days, caused a strong reduction of BAMs on day 3 after the last injection, as assessed by immunofluorescence and cell counting. Control animals were wild type mice that received DTx injection at the same time points. On day 3 after the last DTx administration, ischemia was induced by middle cerebral artery occlusion (tMCAo) for 45 min followed by 24h reperfusion. At 24h, mice underwent an MRI scan and the brain was processed for fluorescence activating cell sorting in order to isolate CD31+ EC from the cerebral tissue and perform RNA sequencing. Our preliminary data show that the depletion of BAMs tends to reduce the infarct volume in mice, suggesting that BAMs may play a detrimental role under ischemic conditions. Furthermore, the changes in EC RNA expression in physiological condition and after ischemia suggest that BAMs play a role in the BBB integrity. We are currently analyzing more in detail the data in order to find the biological significance of these changes.

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## P.2. SPHINGOSINE KINASE 2 DEFICIENCY WORSENS THE STROKE OUTCOME IN MICE

**Pedragosa J**, Gallizioli M, Planas AM

Department of Neuroscience and Experimental Therapeutics, Institute for Biomedical Research of Barcelona (IIBB), Spanish National Research Council (CSIC), IDIBAPS, Barcelona, Spain.

Background: Sphingosine kinases phosphorilate sphingosine to sphingosine 1-phosphate (S1P) to keep balance in the metabolites of sphingolipids. S1P is important for the regulation of lymphocyte trafficking and vascular tone. Sphk2 functions are controversial, however, in a genetic mouse model of deletion of SphK2 but not SphK1, increased ischemic lesion size and worsened neurological function are observed after a transient model of brain ischemia. The objective of this study was to investigate the contribution of Sphk2 to the lymphocte functions and trafficking to the ischemic lesion.

Methods: Permanent ischemia was induced in adult Sphk2-/- and C57BL6/N mice of both sexes. Immune cell populations from cervical lymph nodes, blood and brain were studied by flow cytometry and qRT-PCR at days 1, 4 and 7 post ischemia. We studied the brain lesion by T2w-MRI and assessed functional outcome with behavioral tests.

Results: Sphk2 deficiency reduces the plasma levels of S1P and increases the retention of CD8+ CD69+ lymphocytes in the brain and cervical lymph nodes. In the brain of Sphk2 mice after stroke the resident T cells (CD8+ CD69+) contibute to the inflammatory status with higher production of interferon gamma. In addition, Sphk2 deficiency increases the size of the brain lesion, worsens the neurological function and increases the overall inflamatory response, as measured by qRT-PCR of brain cortical tissue (Cd69, Il1b, Arg1, Irg1 and Mmp3).

Conclusions: Brain resident T cells (CD8+ CD69+) retained in the brain by the absence of Sphk2 contribute to the worsening of stroke outcome in mice by inducing local inflammation.

*Funded by MICINN (PID2020-113202RB-I00)*

## P.3. INTERFERON PATHWAYS ACTIVATION IN MICROGLIA OF MICE AND HUMANS AFTER CEREBRAL ISCHEMIA

**Gallizioli M**[1], Arbaizar-Rovirosa M[1], Pedragosa J[1], Planas AM[1]

Department of Neuroscience and Experimental Therapeutics, Institute for Biomedical Research of Barcelona (IIBB), Spanish National Research Council (CSIC)

Brain ischemia causes necrotic cell death, microglia reactivity and blood-brain barrier breakdown. Various types of danger signals are released from injured cells and trigger immune responses mediated by pattern recognition receptors (PRRs). Microglial cells are equipped with several PRRs that can ultimately induce inflammation. Our aim was to investigate the inflammatory response of microglia to ischemia in mice and humans, focusing on interferon (IFN) pathways. We induced a transient cerebral ischemia in mice by middle cerebral artery occlusion followed by reperfusion. We performed RNA-Seq analysis comparing the transcriptomic profile of microglia isolated by fluorescence activated cell sorting from the brain of healthy and ischemic mice. Enrichment analysis showed a strong innate immune response induced by ischemia in microglia, highlighted by upregulation of type-I interferons including Ifnb and several IFN-stimulated genes (ISG). Accordingly, analysing the whole brain tissue, ischemia increased Ifnb, Ifna7 and Ifna9 mRNA expression, as well as many ISG, including Dhx58, Cxcl10, Irf7, and Irg15. The expression of ISG in the brain increases from about 16h post-ischemia, reaching a plateau at 4-7 days, according to a time-course study in brain tissue samples obtained between 1h and 7 days post-ischemia. We also detected enhanced expression of ISG in post-mortem human brain tissue of ischemic stroke patients. To find out the contribution of microglia to ISG expression, we depleted microglia in mice with a CSF1R inhibitor (PLX5622). Ischemia-induced expression of Ifnb, Ifna7, or Ifna9 mRNA was not reduced after microglia depletion, implying that cells other than microglia are the main source of IFNs in the injured brain. However, we found that microglia depletion reduced cerebral ISG expression. Our results show that type I IFNs generated after ischemia activate their receptors in microglia, inducing ISG expression and triggering a specific transcriptional program in these cells.

*Supported by MICINN (PID2020-113202RB-I00) and CSIC (PTI+ Neuroaging platform).*

## P.4. REPOPULATED MICROGLIAL CELLS AFTER DEPLETION IN OLD MICE DESPITE MAINTAINING THE AGING FEATURES ARE PROTECTIVE IN BRAIN ISCHEMIA THROUGH OTHER MECHANISMS

**Arbaizar-Rovirosa M**[1], Pedragosa J[1], Lozano J[3], Casal C[1] , Gallizioli M[1,2],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] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.
[3] Bioinformatics platform, Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain.

Microglial cells invade the brain at embryonic stages and live for long with negligible replacement by peripheral cells under steady state. Microglia of the aged brain manifest signs of dysfunction such as a differential transcriptional profile. Impaired microglial function may contribute to worsen the neurological outcome following stroke in the elderly. Treatment with colony-stimulating factor-1 receptor antagonists enable transient microglia depletion that can be followed by microglia repopulation after interruption of the treatment, causing no known harm to mice. Using this strategy, we aimed to restore microglia function and ameliorate stroke outcome in aged mice.

We used C57BL/6 mice aged 3 months or 22 months. Seven days after interruption of the diet, we induced brain ischemia to the mice by 45 min- middle cerebral artery occlusion followed by reperfusion. We studied the mice at day 4 post-ischemia by assessing the neurological function using a test (Neuroscore), and monitored the extent of the brain lesion by MRI. We also carried out behavioural studies for 15 days post-ischemia. We also generated chimeric mice to investigate the origin of repopulating microglia which proved their derivation from brain cells and not from peripheral hematopoietic cells. We found that the transcriptomic profile of microglia after ischemia was different in old versus young mice. However, repopulated microglia in old mice acquired the same transcriptional profile as the original microglia.

Cerebral ischemia/reperfusion induces strong innate immune responses in microglia, together with metabolic perturbances and lipid droplet biogenesis in young mice. In aged mice, a subset of microglia accumulates lipid droplets under steady state and displays exacerbated innate immune responses after stroke. Microglia renewal in old mice reduces the lipid droplet content and improves the neurological outcome of stroke. This study shows that age-dependent lipid droplet-enriched microglia contribute to impair stroke outcome in old mice.

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## P.5. THE NEUROPROTECTION AFFORDED BY ASTROCYTIC CREB IN EXPERIMENAL TRAUMATIC BRAIN INJURY IS ASSOCIATED WITH REGULATION OF LACTATE AND LIPID METABOLISM

**Fernández-González I**[1], Eraso-Pichot A[1], Jové M[2], Portero-Otín M[2], Wood L[3], Giralt M[1], Hidalgo JM[1], Pardo L[1,4], Galea E[1,5]

[1] Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra
[2] Institut de Recerca Biomèdica, Universitat de Lleida, Spain
[3]Georgia Institute of Technology, Atlanta, Georgia, USA
[4] Current address: Beatson Institute for Cancer Research, Glasgow, United Kingdom.
[5] ICREA, 08010 Barcelona, Spain.

The clinical challenge in traumatic brain injury (TBI) is to halt the delayed neuronal loss that happens hours and days after the insult. We report that the targeted activation of the transcription factor cAMP-response element binding protein (CREB) in reactive astrocytes prevents secondary injury in an experimental model of TBI. However, the mechanisms underlying protection remain unknown. Previous results from metabolomics performed in cerebral cortex of wild type controls and Gfa2-tTA/TetO-VP16CREB bitransgenic mice, in which the constitutively active CREB construct is expressed in astrocytes, revealed increased production of lactate from glycolysis and changes in lipid metabolism. So, our main hypothesis is that astrocytic CREB mediated neuroprotection is due to increased lactate action and crosstalk between astrocytes and neurons, thus rescuing injured brain bioenergetics. Here examined the effect of lactate action using α-cyano-4-hydroxy-cinnamic acid (4CIN), a small-molecule inhibitor of lactate transport, through subcutaneous Alzet osmotic pumps implanted bitransgenic and wild type mice, subjected to cryolesions. As lactate participates in our neuroprotection pathway and it is an emerging neuroprotectant, we expect a disruption of this effect due to its uptake inhibition in our TBI model (results are pending to be analyzed).

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## P6. RTP801 REGULATION OF NEUROINFLAMMATION

**Chicote González A**[1,2,3], Solana Balaguer J[1,2,3] , Campoy Campos G[1,2,3], Pérez Sisqués L[1,2,3,4] , Malagelada C[1,2,3]

[1] Institut de Neurociències, School of Medicine and Health Sciences, Barcelona, Spain
[2] Universitat de Barcelona, Biomedicine, Barcelona, Spain
[3] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, (CIBERNED), Madrid, Spain
[4] King's College London, Centre for Craniofacial and Regenerative, London, United Kingdom
[3] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, (CIBERNED), Madrid, Spain
[4] King's College London, Centre for Craniofacial and Regenerative, London, United Kingdom

Neuroinflammation is a key player in many neurodegenerative diseases where the crosstalk between neurons, astrocytes, and microglia is crucial. The release of pro-inflammatory molecules can have devastating consequences, as it leads to synaptic dysfunction, astro and microgliosis and neuronal death.

RTP801 is encoded by the stress-sensitive gene DDIT4 and is upregulated several neurodegenerative disorders, including Parkinson, Alzheimer and Huntington's disease. The main function of RTP801 is to inactivate the mammalian target of rapamycin (mTOR), among others. We recently found that RTP801 is up regulated in AD patients' hippocampus. and silencing neuronal RTP801 prevented cognitive impairment and astro and microgliosis in the 5xFAD AD mouse model. Hence, the aim of this study is to investigate the mechanism by which RTP801 mediates the inflammatory response in astrocytes and microglia. In the RTP801 KO mouse NLRP1, an effector of the inflammasome, were higher in comparison to WT. However, we did not find differences in the number of astrocytes or microglia. Interestingly, other components of the inflammasome remain similar. Notwithstanding, in neuronal and astrocyte primary cultures, silencing RTP801 reduced the levels of NLRP1 but did not change the effect of LPS treatment. Hence, our data suggest that RTP801 is somehow regulating NLPR1 levels. Investigating the mechanisms by which RTP801 regulates neuroinflammation will lead to a better understanding of neurodegenerative diseases.

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## P.7. MACROPHAGES MAY PHAGOCYTOSE WASTEOSOMES (CORPORA AMYLACEA) THROUGH DIFFERENT PATHWAYS

**del Valle J**[1,2,3], Riba M[1,2,3], Campo-Sabariz J[1,4], Tena I[1], Romera C[1], Molina-Porcel L[5,6], Ximelis T5,6, Calvo M[7], Ferrer R[1,4], Martín-Venegas R[1,4], Vilaplana J[1,2,3] and Pelegrí C[1,2,3]

[1] Secció de Fisiologia, Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Barcelona.
[2] Institut de Neurociències, Universitat de Barcelona, Barcelona.
[3] Centros de Biomedicina en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid.
[4] Institut de Recerca en Nutrició i Seguretat Alimentàries (INSA-UB), Universitat de Barcelona, Barcelona.
[5] Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona.
[6] Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS, Barcelona.
[7] Unitat de Microscòpia Òptica Avançada - Campus Clínic, Facultat de Medicina, Centres Científics i Tecnològics - Universitat de Barcelona, Barcelona.

Corpora amylacea, lately renamed as wasteosomes, are age-related granular structures formed primarily by polymerized hexoses that appear during aging and also accumulate in specific areas of the human brain in neurodegenerative conditions. Recent studies indicate that these structures entrap residual products of different origins. As they are expelled from the brain to the cerebrospinal fluid (CSF) and are thereafter phagocytosed by macrophages, they may act as containers that remove waste producte from the brain. In the present study, we analyze the different mechanisms involved in the phagocytosis of wasteosomes. We purified wasteosomes from human CSF and incubated them with THP-1 macrophages. Immunofluorescence staining techniques were later performed to evaluate the mechanisms involved in their phagocytosis. We also immunostained human hippocampal sections to study the interactions between wasteosomes and macrophages at central nervous system interfaces. Our results indicated that the phagocytosis by THP-1 macrophages can be triggered through the CD206 and the CD35 receptors, but not the FAIM3 receptor, whereas all these receptors may be involved, in vivo, in the phagocytosis of wasteosomes at central nervous system interfaces or beyond. Moreover, we observed that the wasteosomes obtained from the CSF are opsonized by MBL and the complement protein C3b, and can also contain mannose or other targets of CD206. All these observations indicate that, in vivo, different mechanisms may be involved in the phagocytosis of wasteosomes, some of them triggering non-inflammatory responses and avoiding tissue damage, supporting the role of the immune system in the elimination of wasteosomes.

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## P.8. MACROPHAGES CAN DIGEST HUMAN BRAIN WASTEOSOMES (CORPORA AMYLACEA)

**Riba M**[1,2,3], del Valle J[1,2,3], Campo-Sabariz J[1,4], Tena I[1], Romera C[1], Molina-Porcel L[5,6], Ximelis T[5,6], Calvo M[7], Ferrer R[1,4], Martín-Venegas R[1,4], Pelegrí C[1,2,3] and Vilaplana J[1,2,3]

[1] Secció de Fisiologia, Departament de Bioquímica i Fisiologia, Universitat de Barcelona, Barcelona.
[2] Institut de Neurociències, Universitat de Barcelona, Barcelona.
[3] Centros de Biomedicina en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid.
[4] Institut de Recerca en Nutrició i Seguretat Alimentàries (INSA-UB), Universitat de Barcelona, Barcelona.
[5] Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona.
[6] Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS, Barcelona.
[7] Unitat de Microscòpia Òptica Avançada - Campus Clínic, Facultat de Medicina, Centres Científics i Tecnològics - Universitat de Barcelona, Barcelona.

Corpora amylacea, also known as wasteosomes, are spherical polyglucosan bodies that accumulate primarily in the periventricular, perivascular and subpial regions of the human brain during aging and some neurodegenerative diseases. Wasteosomes, acting as waste containers, collect waste substances of different origins, are expelled from the brain to the cerebrospinal fluid (CSF) and are thereafter phagocytosed by macrophages. In the present study, we analyze the phagocytosis of wasteosomes and the mechanisms involved in this process. We purified wasteosomes from human CSF and stained them with Concanavalin A-rhodamine, AF555-NHS probe or with PAS staining. Then, they were incubated with THP-1 macrophages stained with the Vybrant® CFDA-SE Cell Tracer Kit. Time-lapse recording techniques were performed to evaluate the phagocytosis process. We observed that THP-1 macrophages phagocytose and process wasteosomes. We also observed that once phagocytosed, Concanavalin A-rhodamine-labelled and AF555-NHS-labelled wasteosomes were digested or fragmented and the fluorescent protein fraction was exposed on the surface of macrophages as well as transferred from one macrophage to another. We observed a lower reactivity of macrophages towards PAS-stained wasteosomes. In any case, these time-lapse studies revealed that under all the experimental conditions THP-1 macrophages phagocytose or interact with wasteosomes. Our findings support the role of the immune system in the elimination of wasteosomes.

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## P9. EXPLORING ASTROCYTE HETEROGENEITY IN THE AGING MOUSE HIPPOCAMPUS

**Casares-Crespo L**, Franch-Ibáñez C, Mira H

Instituto de Biomedicina de Valencia (IBV-CSIC)

Astrocytes are vital for the proper function of the central nervous system (CNS). They perform many essential roles such as blood-brain barrier and synapse formation, provide trophic support to neurons and modulate synaptic transmission. In recent years, it has been stated that astrocytes exhibit functional and molecular heterogeneity between and within brain regions.

The purpose of this study was to assess gene expression profiles of hippocampal astrocyte subtypes defined by surface expression of the astrocyte markers ACSA-1/GLAST/SLC1A3 and ACSA-2/ATP1B2 during aging. We used wild type mice (C57BL/6JRccHsd strain) of 2 and 18 months of age, microdissected the hippocampus and dissociated it with the Neural Tissue Dissociation Kit (Miltenyi). Following myelin and red blood cells removal, we stained the resulting cell suspension with ACSA-1-PE and ACSA-2-FITC antibodies. We identified three distinct astrocyte subpopulations by flow cytometry: the ACSA-1+ (A-/G+), ACSA-2+ (A+/G-) and ACSA-1+/ACSA-2+ (A+/G+) cells. Bulk RNA-seq transcriptome analysis showed that 4302 and 1556 genes were differentially expressed ($p \leq 0.05$) between A+/G- and A+/G+ populations, respectively, with age. Expression of the ApoE gene related with cholesterol transport increased with age in A+/G- astrocytes. This change has also been reported in aging astrocytes from other parts of the mice brain, such as cerebellum (Boisvert et al. 2018). On the other hand, Lig3 and Xrcc4 showed lower expression in A+/G+ astrocytes with age. Both Lig 3 and Xrcc4 genes encode DNA repair-related proteins and are repressed in human senescent astrocytes induced by X radiation compared to control ones (Limbad et al. 2020). Ongoing functional assays employing conditioned media from cultured hippocampal astrocytes isolated from 2- and 18-month-old animals will be presented and discussed.

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## NEURODEVELOPMENT AND RELATED DISEASES

### P.10. NOVEL GENETIC PLAYERS IN SUBCELLULAR BRANCHING AND CELL GUIDANCE

**De La Torre L**[1,2] , Araújo S[1,2]

[1] Department of Genetics, Microbiology and Statistics. University of Barcelona. Av.Diagonal 643, 08028 Barcelona, Spain
[2] Institute of Biomedicine of the University of Barcelona (IBUB)

Embryonic development implies a great deal of morphogenetic changes, leading to the complete formation of an individual from a fertilized egg. Each of its organs are formed throughout these stages by different cellular processes. Migration and branching processes are important processes involved in nervous and tracheal system formation. How cells branch or migrate at the correct time and place and what are the molecular mechanisms implicated are essential for the modulation of this cell behaviour during development and regeneration, as branching and cell guidance processes require a precise temporal and spatial coordination. The aim of this project is the understanding of different cell branching and migration phenotypes in the tracheal and nervous systems respectively, in order to unveil new molecules involved in these processes during development. We selected D. melanogaster mutants displaying phenotypes in the nervous and tracheal systems. We focused on a gene located in the mutant region, called teiresias, a key candidate in generating these mutant phenotypes. Here we describe a variety of experiments designed to answer which are the molecular and cellular mechanisms involved.

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## P.11. THE ROLE OF GKT IN DROSOPHILA MELANOGASTER NERVOUS SYSTEM DEVELOPMENT

**Díaz N**[1,2], Araújo S.J[1,2]

[1] Department of Genetics, Microbiology and Statistics. University of Barcelona
[2] Insitute of Biomedicine of the University of Barcelona (IBUB)

A range of different types of DNA repair deficiencies can lead to both nervous system and developmental problems. Spinocerebellar ataxia with axonal neuropathy type 1 (SCAN-1) is a debilitating peripheral neuropathy characterized by slowly progressive cerebellar ataxia and atrophy. All SCAN-1 patients identified to date carry the same neomorphic active site mutation in tyrosyl-DNA phosphodiesterase 1 (TDP1). Human TDP1 is required for repair of chromosomal strand breaks that arise from abortive topoisomerase 1 (TOP1) reactions. Like in humans, the homologue of TDP1 in Drosophila, a gene named glaikit (gkt) encodes a tyrosyl-DNA phosphodiesterase 1. The alignment of the aminoacidic sequences encoded by TDP1 and gkt reveals that the catalytic domain of the enzymes is conserved between H. sapiens and D. melanogaster. Glaikit was found to be essential for the formation of epithelial polarity and nervous system development. The fact that the Drosophila homologue appears to have different functions, may help elucidating the complex aetiology of SCAN1. Our aim is to elucidate the function of gkt in the nervous system of Drosophila melanogaster. To study it, we are using the null mutant gktG85 and characterizing the mutant phenotype by analysing the embryonic CNS and PNS.

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## P.12. ROLE OF CENTROSOMES IN AXON GUIDANCE OF THE MOTOR NEURONS IN DROSOPHILAMELANOGASTER

**Sellés J**[1,2] , Araújo SJ[1,2]

[1] Department of Genetics, Microbiology and Statistics. University of Barcelona
[2] Institute of Biomedicine of the University of Barcelona (IBUB)

One of the most interesting processes during nervous system development is the establishment of stereotypic neuronal networks. An essential step in this process is the outgrowth and precise navigationof axons towards their synaptic partner cells. The growth cone is a unique structure capable of guiding axons to their proper destinations. Within the growth cone, extracellular guidance cues are interpreted and then transduced into physical changes in the actin filament and microtubule cytoskeletons. Is a mechanism that is part of the morphogenic process. The formation, stabilization and remodelling of axons is achieved in response to extensive microtubule dynamics. Centrosomes are the main microtubule organizing centres (MTOC) and because microtubules regulate cell shape, centrosomes are involved in different cellular branching and migration processes. The full link between the number of centrosomes and the axon pathfinding is not well known yet. Given that the formation process of motorand muscular systems is interconnected, we used the Drosophila neuromuscular model to provide evidence that embryos with less and more centrosomes have axon guidance and muscle morphology problems. Moreover, we report that musculature with altered number of centrosomes has a non- autonomous effect on axon guidance of motor neurons. Our results demonstrate the importance of centrosomes in mediating microtubule remodelling during early axon development in Drosophila melanogaster motor neurons.

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## P.13. A ROADMAP FOR POSTNATAL BRAIN MATURATION: CHANGES IN WHITE AND GREY MATTERCOMPOSITION DURING DEVELOPMENT

**Peris M**[1], Zerpa O[1], Manich G[1], Almolda B[1], Benseny-Cases N[2], Perálvarez-Marín A[2], González B[1],Castellano B[1]

[1] Institut de Neurociències, Departament de Biologia cel·lular, fisiologia i immunologia, Universitat Autònoma de Barcelona, Bellaterra.
[2] Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra.

Synchrotron based Fourier Transform Infrared microspectroscopy (µFTIR) is a technique used to analyze biochemical properties of biological samples. Specifically, the study of lipid/protein ratio, lipid oxidation and protein structure are parameters frequently studied in pathological (such as Alzheimer's) and non- pathological processes (such as ageing) using this technique. However, there are no studies of this type in the normal development of the central nervous system (CNS). Here we show a first detailed roadmap of the biochemical composition of the CNS during postnatal development in mice. In this study, we describe changes in lipid and protein composition from postnatal day 0 (P0) to P28, and we compare white and grey matter areas of mouse brain and cerebellum (strain C57BL/6). We found that, at birth, differences between white and grey matter were minimal, and were enhanced during development. In white matter, the presence of lipids was greater than in grey matter from P14 to adulthood, and these were poor in unsaturated olefinic and carbonyl groups. On the other hand, we wanted to check the correlation of other known myelin study techniques with µFTIR, such as histochemical and immunohistochemical stainings. In this way, compared to µFTIR, Luxol Fast Blue and Oil Red O lipid stainings reflected changes observed in white matter myelination better than Sudan Black lipid staining. However, MBP and MOG immunohistochemical stainings did not reflect so well changes in the composition of white matter compared to µFTIR, but they did reflect the modifications in the composition of the secondary structure of the proteins (alfa-helix and beta-sheet) present in the sample.Furthermore, consistent with the literature, the primary myelination process begins earlier in the cerebellum than in the brain. Our results lay a foundation for future studies in developmental diseases with the µFTIR technique, such as autism or perinatal white matter injury (WMI).

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## NEURODEGENERATIVE DISEASES

### P.14. THE GUT-BRAIN AXIS IN A NOVEL HUMANIZED TRANSGENIC MOUSE MODEL FOR PARKINSON´S DISEASE AND BRAIN AGING

**Lorente-Picón M**[1], Laguna A[1], Vila M[1,2,3]

[1] Vall d'hebron Research Institute (VHIR), Neurodegenerative Diseases, Barcelona, Spain
[2] Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
[3] Universitat Autònoma de Barcelona, Barcelona, Spain

Aim: 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.

Methods: To clarify how GI dysfunction is involved in disease pathogenesis and/or in modulating the manifestation of PD symptoms, we characterized the GI function and the key mechanisms involved in the gut-brain axis of a new humanized transgenic PD mouse model (Tg-Th-hTyr) that progressively accumulates neuromelanin in all catecholaminergic nuclei of the brain, including the dorsal motor nucleus of the vagus nerve. We have performed a battery of motor and non-motor behavioral tests to assess the phenotype of these animals, including the GI function. In addition, we have evaluated gut dysbiosis in fecal samples by 16S RNA gene sequencing and metabolomics of Tg and wild-type (wt) littermates.

Results: show impaired motor activity in Tg mice compared to wt at 6 months of age. We also detected increased fecal output in Tg mice placed in a novel environment, suggesting alterations in the hypothalamic-pituitary-adrenal (HPA) axis. We also observed a significant increase in body weight and water/food intake in Tg mice.

Conclusions: Our results indicate that the gut-brain axis is altered in our PD mice model and that this model can contribute to clarify the role of gut dysfunction in PD pathogenesis.

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## P.15. ANALYSIS OF GENETIC RISK FACTORS RELATED TO PARKINSON'S DISEASE IN PATIENTS WITH MYASTHENIA GRAVIS USING A WHOLE EXOME SEQUENCING

**Zalba-Jadraque L**[1], Salvadó M[1,2,4], García-Carmona C[1], García-Rodríguez C[1], González V[3], Pérez-Castro JA[1], Juntas-Morales R[1,2,4], Vidal-Taboada JM[1]

[1] Vall d'Hebron Institut de Recerca, Laboratorio Sistema Nervioso Periférico, Barcelona.
[2] Hospital Universitario Vall d'Hebron, Departamento de Neurología, Unidad de Enfermedades Neuromusculares, Barcelona.
[3] Hospital Universitario Vall d'Hebron, Departamento de Neurología, Unidad de Trastornos del movimiento, Barcelona.
[4] EURO-NMD: European Reference Network for Rare Neuromuscular Diseases

Myasthenia gravis (MG) is a rare, heterogeneous and multifactorial autoimmune disease, mainly characterized by fluctuating weakness and muscular fatigability affecting ocular, bulbar and/or limb muscles. In the Neuromuscular Diseases Unit at the HUVH, we identified 10 cases of patients diagnosed with MG and Parkinson's disease (PD) and 18 cases with MG and Parkinsonism. The association between PD and MG is uncommon. HLA-DR/DQ haplotypes and non-HLA genes have been proposed as major genetic susceptibility factors and phenotype modifiers in both MG and PD. Objective: To identify genetic variants in the HLA cluster (HLA-A, HLA-B, HLA-DQ, HLA-DR) and in other Parkinson's-related genes in a cohort of MG patients. Methodology: Whole Exome Sequencing analysis was performed in 100 unrelated sporadic MG patients. Bioinformatic analysis and variant priorisation were conducted following an in-home pipeline and the DNASTAR software, Nexus-SNP, Ensembl VEP and Varsome tools. Results: A total of 293,416 genetic variants were identified, in which 1,337 were located in PD-related genes. Of them, 429 showed a higher frequency in MG patients when compared to the general population. 88 were non-synonymous variants: 31 were predicted as deleterious/damaging by functional prediction tools and considered as pathogenic in ClinVar. 13 variants were linked to the physiopathology of PD according to GWAS studies. 19 variants were located in splicing regions and 40 in the HLA-DQB1 locus. Conclusion: These preliminary results suggest the existence of a possible common genetic architecture between MG and PD and the probable role of autoimmunity in the etiology of PD. Eventually, 36 variants have been selected for further analysis.

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## P.16. NEURONAL INDUCTION AND BIOENERGETICS CHARACTERIZATION OF HUMAN FOREARM ADIPOSE STEM CELLS FROM PARKINSON'S DISEASE PATIENTS AND HEALTHY CONTROLS

**Morén C**[1,2,3], González-Casacuberta I[1,2,3], Cardellach F[1,2,3]

[1] Cellex-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine and Health Science, University of Barcelona, Spain.
[2] Internal Medicine Department, Hospital Clínic of Barcelona, Barcelona, Spain.
[3] Biomedical Research Networking Center on Rare Diseases (CIBERER), Madrid, Spain.

Neurodegenerative diseases, such as Parkinson's disease, are heterogeneous disorders with a multifactorial nature involving impaired bioenergetics. Stem-regenerative medicine and bioenergetics have been proposed as promising therapeutic targets in the neurologic field. The rationale of the present study was to assess the potential of human-derived adipose stem cells (hASCs) to transdifferentiate into neuronal-like cells (NhASCs and neurospheres) and explore the hASC bioenergetic profile. hASC neuronal transdifferentiation was performed through neurobasal media and differentiation factor exposure. High resolution respirometry was assessed. Increased MAP-2 neuronal marker protein expression upon neuronal induction ($p<0.05$ undifferentiated hASCs vs. 28-36 days of differentiation) and increased bIII-tubulin neuronal marker protein expression upon neuronal induction ($p<0.05$ undifferentiated hASCs vs. 6-28-36 days of differentiation) were found. The bioenergetic profile was detectable through high-resolution respirometry approaches in hASCs but did not lead to differential oxidative capacity rates in healthy or clinically diagnosed PD-hASCs. We confirmed the capability of transdifferentiation to the neuronal-like profile of hASCs derived from the forearms of human subjects and characterized the bioenergetic profile. Suboptimal maximal respiratory capacity trends in PD were found. Neuronal induction leading to positive neuronal protein expression markers is a relevant issue that encourages the suitability of NhASC models in neurodegeneration.

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## P.17. MALE SEX BIAS IN PARKINSON'S DISEASE IS LINKED TO AN ACCELERATED AGE-DEPENDENT NEUROMELANIN ACCUMULATION

**Guillard-Sirieix C**[1], Romero-Giménez J[1], Cuadros T[1], Parent A[1], Laguna A[1], Vila M[1,2,3]

[1] Vall d'Hebron Research Institute (VHIR)-Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain
[2] Autonomous University of Barcelona, Cerdanyola del Vallès, Barcelona, Spain
[3] Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Men have a higher incidence and prevalence of Parkinson's disease (PD), earlier disease onset, more severe motor symptoms and progression, and more frequent cognitive decline compared to women. However, most PD studies do not consider the influence of sex, thus the molecular mechanisms underlying sex differences in PD remain unknown. Sex steroids modulate dopaminergic pathways, in both normal and pathological states, and estrogens improve PD symptoms in both men and women. Estrogens are also able to modulate melanin production in the skin and we have recently reported, in both humans and experimental animals, that excessive age-dependent intracellular neuromelanin accumulation above a pathogenic threshold triggers PD pathology. Here we assessed whether differences in neuromelanin production/accumulation could underlie the differential effect of sex on PD. First, using postmortem human brain tissue, we found that intracellular neuromelanin levels within nigral dopaminergic neurons from age-matched control subjects are significantly higher in men than in women and that men reach earlier the pathogenic threshold of neuromelanin accumulation, even in absence of PD. We then assessed the effect of sex on the only rodent model currently available of age-dependent neuromelanin production within PD-vulnerable neurons, based on the viral vector-mediated expression of melanin-producing enzyme tyrosinase (AAV-hTyr) in the substantia nigra of rats. This model, developed by our group, exhibits major PD features in parallel to progressive neuromelanin accumulation. We observed that AAV-hTyr-injected male rats exhibit an earlier and greater accumulation of neuromelanin compared to female animals, reaching earlier the pathogenic threshold of intracellular neuromelanin accumulation. Remarkably, ovariectomized (OVX) female rats injected with AAV-hTyr accumulated neuromelanin more rapidly than non-OVX female animals and ultimately reached pathological neuromelanin levels similar to their male counterparts. These results suggest that an increased/accelerated accumulation of neuromelanin in men across life may underlie their higher risk to develop PD, compared to women.

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## P.18. SEX DIFFERENCES IN MOUSE MODEL OVEREXPRESSING HUMAN α-SYNUCLEIN IN SEROTONIN NEURONS: ENDOPLASMIC RETICULUM STRESS AND KETAMINE EFFECT

**Sarriés-Serrano U**[1,2,3], Miquel-Rio L[1,2,3], V. Paz V[1,2,3], Meana JJ[3,4,5], Bortolozzi A[1,2,3]

[1] Institute of Biomedical Research of Barcelona (IIBB), Spanish National Research Council (CSIC),Barcelona
[2] August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona
[3] Mental Health Networking Biomedical Research Centre (CIBERSAM), ISCIII, Madrid
[4] Department of Pharmacology, University of the Basque Country, Leioa
[5] Biocruces Bizkaia Health Research Institute, Barakaldo

Aims**:** Anxiety/depression are the most prevalent neuropsychiatric disorders in Parkinson's disease (PD)patients (50%), occurring at different stages and with different affection in men and women. Neuropathological and functional changes in the serotonin (5-HT) system are involved in the PD prodromal phase and contribute to non-motor symptoms. Using a mouse model of human α-synuclein (h-α-Syn) overexpression in 5-HT neurons, we aim to evaluate: 1- behavioral phenotype, 2- endoplasmicreticulum (ER) stress and unfolded protein response (UPR) activation, 3- possible sex differences and 4- acute effects of ketamine and citalopram antidepressants on behavioral phenotype.

Methods:Recombinant AAV vector serotype 5 (AAV5) encoding wild-type h-α-Syn was used to overexpress h-α- Syn in raphe 5-HT neurons of male and female mice. The behavioral phenotype was examined at 4 weeks post-infusion. Immunohistochemistry was performed for h-α-Syn. Protein levels of UPR pathwaymarkers (BIP, GRP94, p-eIF2α, and p-eEF2) and BDNF were assessed by Western-blot. Statistical significance was ascertained by t-tests or one-way ANOVA, as appropriate.

Results: In male mice, raphe h-α-Syn overexpression induced a depressive-like phenotype in tail suspension andforced swim tests, and reduced raphe BDNF levels. In parallel, significant increases in BIP and GRP94 levels were detected indicating UPR pathway activation. ER stress also increased p-eIF2α and p-eEF2 levels, suggesting PERK pathway activation. However, female mice exhibited anhedonia in the sucrose preference test and an anxiety-like phenotype in the dark-light box test and preliminary results in females do not evidence an activation of UPR pathway.

Conclusions:Overexpression of h-α-Syn in the mouse 5-HT system induces a sex-specific behavioral phenotype and UPR activation, suggesting that different brain circuits may be affected and replicating the outcomes reported in PD patients.

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## P.19. HUMAN ALPHA-SYNUCLEIN OVEREXPRESSION IN MOUSE SEROTONIN NEURONS ELICITS A DEPRESSIVE-LIKE PHENOTYPE: FOCUS ON BRAIN CONNECTIVITY AND SYNAPTIC DENSITY

**Paz V** [1,2,3], Miquel-Rio L[1,2,3], Torres-López M[1,2], Casal C[1], Muñoz-Moreno E[4], López-Gil X[4], Bortolozzi A[1,2,3]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), Spanish National Research Council (CSIC), Barcelona, Spain

[2] Institut d'Investigacions August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[3] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid, Spain

[4] Magnetic Resonance Imaging Core Facility, Institut d'Investigacions August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Objectives: Besides the motor symptoms that define Parkinson's disease (PD), up to 50% of patients experience cognitive decline and psychiatric disorders, among which anxiety and depression are the most prevalent neuropsychiatric symptoms. Although dopamine system deficits are involved in several non-motor manifestations, structural and functional alterations in the serotonin (5-HT) system also occur in PD and may contribute to non-motor phenotypes. In this study, we investigated how α-synucleinopathy in the 5-HT system triggers synaptic alterations in the brain circuits involved in emotional and mood control.
Methods: We used a new mouse model of α-synucleinopathy in the 5-HT system, based on AAV5-induced overexpression of wild-type human-α-synuclein (h-α-Syn) in raphe nuclei. Mice were assessed at 4 and 8 weeks later. Cytoskeletal components and synaptic vesicle SV-associated proteins were examined by confocal microscopy. Brain functional connectivity was analyzed in the resting state (rsfMRI) by BOLD signal. The cellular activity was measured by Egr-1 mRNA expression in different brain regions.
Results: Overexpression of h-α-Syn in 5-HT neurons leads to progressive reductions of MAP-2 density in different projection brain regions, including prefrontal, cingulate and motor cortices, caudate-putamen, etc. Simultaneously, h-α-Syn mice also showed changes in SV2A and synaptophysin levels in some of the analyzed brain regions. Hypoconnectivity in caudate-putamen and hippocampus, as well as increased Egr-1 mRNA expression, were detected 8-weeks later.
Conclusions: These data indicate that presynaptic h-α-Syn accumulation in 5-HT neurons causes alterations in crucial components for the synaptic structure and function in circuits involved in emotional and mood control in PD.

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## P.20. CROSSTALK BETWEEN α-SYNUCLEIN AND NEUROMELANIN EXACERBATES PARKINSON'S DISEASE PATHOLOGY IN MELANIZED TYROSINASE-EXPRESSING RODENTS

**Nicolau-Vera A**[1], Cuadros T[1], Romero-Giménez J[1], Parent A[1], Cladera-Sastre JM[1], Laguna A[1], Vila M[1,2,3]

[1] Vall d'Hebron Research Institute (VHIR)-Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain

[2] Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain

[3] Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Parkinson's disease (PD) is characterized by a preferential loss of neurons that contain the pigment neuromelanin, especially dopaminergic neurons of the substantia nigra (SN), and the presence in affected neurons of α-synuclein (αSyn)-containing insoluble cytoplasmic aggregates termed Lewy bodies (LB). While αSyn aggregation is considered a central pathogenic event in PD, the mechanisms and significance of LB formation remains unknown. In PD brains, LBs appear in close physical association with neuromelanin within affected neurons. In addition, it has been reported that αSyn redistributes to the lipid component of neuromelanin at early PD stages and that αSyn is entrapped within neuromelanin granules extracted from PD, but not control, brains. The increased concentration of neuronal αSyn and neuromelanin pigment in SN neurons may predispose these neurons to LB formation and cell death. However, it has not been possible yet to experimentally assess in vivo a potential pathological interaction between αSyn and neuromelanin because, in contrast to humans, neuromelanin is absent in common experimental animals such as rodents. We recently developed the first rodent model of human-like neuromelanin production based on the viral vector-mediated nigral expression of melanin-producing enzyme tyrosinase (AAV-hTyr). This has revealed that neuromelanin can trigger PD pathology when accumulated above a specific pathogenic threshold. Here we assessed the potential interaction between aSyn and neuromelanin by combining αSyn overexpression with hTyr-induced neuromelanin production in rodents. Compared to regular non-melanized animals, AAV-mediated nigral expression of human αSyn in melanized hTyr-expressing rodents resulted in an increased formation of aSyn oligomeric species within melanized neurons, as assessed by proximity ligation assay (PLA), an enhanced and continuous production LB-like inclusions and an aggravated nigrostriatal denervation. Our results indicate that increased levels of αSyn, as it occurs in PD patients, may accelerate and enhance neuromelanin-linked PD pathology.

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## P.21. HUMAN FRONTAL CORTEX IN ALS-FTLD-TDP43 PROTEINOPATHY SPECTRUM SHOWS LIPID ALTERATIONS THAT ARE PARTLY RELATED TO PEROXISOME IMPAIRMENT

**Martin-Gari M**[1], Andrés-Benito P[2], Mota-Martorell N[1], Obis E[1], Sol J[1], Portero-Otin M[1], Pujol A[3], Ferrer I[2,3], Pamplona R[1], Jové M[1]

[1] Department of Experimental Medicine, University of Lleida-Lleida Biomedical Research Institute (UdL-IRBLleida), 25198, Lleida, Spain.
[2] Center for Biomedical Research On Neurodegenerative Diseases (CIBERNED), Institute of Health Carlos III, 28220, Madrid, Spain.
[3] Department of Pathology and Experimental Therapeutics, University of Barcelona, L'Hospitalet de Llobregat, 08907, Barcelona, Spain.

Peroxisomes play a key role in lipid metabolism, and peroxisome defects have been associated with neurodegenerative diseases such as X-adrenoleukodystrophy and Alzheimer's disease. This study aims to elucidate the contribution of peroxisomes in lipid alterations of area 8 of the frontal cortex in the spectrum of TDP43-proteinopathies. Cases of frontotemporal lobar degeneration-TDP43 (FTLD-TDP), manifested as sporadic (sFTLD-TDP) or linked to mutations in various genes including expansions of the non-coding region of C9ORF72 (c9FTLD), and of sporadic amyotrophic lateral sclerosis (sALS) as the most common TDP43 proteinopathies, were analysed. We used transcriptomics and lipidomics methods to define the steady-state levels of gene expression and lipid profiles. Our results show alterations in gene expression of some components of peroxisomes and related lipid pathways in frontal cortex area 8 in sALS, sFTLD-TDP and c9FTLD. Additionally, we identify a lipidomic pattern associated with the ALS-FTLD-TDP43 proteinopathy spectrum, notably characterised by down-regulation of ether lipids and acylcarnitine among other lipid species, as well as alterations in the lipidome of each phenotype of TDP43 proteinopathy, which reveals commonalities and disease-dependent differences in lipid composition.

Globally, lipid alterations in the human frontal cortex of the ALS-FTLD-TDP43 proteinopathy spectrum, which involve cell membrane composition and signalling, vulnerability against cellular stress and possible glucose metabolism, are partly related to peroxisome impairment.

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## P.22. STRIATAL REDUCTION OF TRANSFERRIN RECEPTOR IN HUNTINGTON'S DISEASE MODELS

**Samperi-Esteve T**[1], Solés-Tarrés I[1], Alberch J[2,3,4], Xifró X[1]

[1] New Therapeutic Targets Group, Departament de Ciències Mèdiques, Facultat de Medicina. Universitatde Girona

[2] Departament de Biomedicina, Institut de Neurociències, Facultat de Medicina, Universitat de Barcelona

[3] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

[4] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)

Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by the expression of mutant Huntingtin (mHTT) protein. HD is characterized by motor dysfunction and the degeneration of striatal medium spiny neurons. Elevated iron levels have been described in the striatum of HD patients and mice models suggesting the iron involvement in the HD pathophysiology. Therefore, we intend to study the role of iron in HD degeneration and to characterize the expression of proteins regulating iron entrance: Transferrin Receptor (TfR) and Divalent Metal Transporter 1 (DMT1). We first added differentconcentrations of ferric ammonium citrate (FAC; from 0.5 to 15 mM) to STHdhQ7/Q7 and STHdhQ111/Q111 cells, a striatal cell line expressing wild-type and mHTT respectively. We observed reduced STHdhQ111/Q111 cell survival compared to STHdhQ7/Q7 cells 24 hours after FAC treatment indicating that expression of mHTT induced more sensibility to iron toxicity. Using the 10mM of FAC, we observed an increase of cleaved caspase-3 in both STHdhQ7/Q7 and STHdhQ111/Q111 cells. Additionally, we detected a reduction of TfR in STHdhQ111/Q111 cells, without changes in DMT1 levels. We also observed a strong downregulation of Iron-responsive element-binding protein 2 (IRP2), a protein that promotes the expression of TfR and that it is degraded at higher iron conditions. We alsomeasured the levels of Glutathione Peroxidase 4 (GPx4) to explore the presence of ferroptosis. STHdhQ111/Q111 cells displayed decreased GPx4 levels than STHdhQ7/Q7 cells. Moreover, the addition of 10mM of FAC exacerbated the GPx4 reduction. Finally, we studied the levels of these proteins in the striatal tissue of R6/1 mice, a HD mice. We observed a reduction of TfR from 12 weeks (onset of symptomatology) to 30 weeks of age (HD late-phase). Altogether, our results support that iron accumulation could accelerate the HD striatal degeneration and we propose an association between irondyshomeostasis and motor symptomatology.

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## P.23. DISTINCT INVOLVEMENT OF DIRECT AND INDIRECT PATHWAYS FROM THE DORSOLATERAL AND DORSOMEDIAL STRIATUM IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE

**Conde-Berriozabal S**[1,2,3], Sitjà-Roqueta L[1,2,3], García-García E[1,2,3], García-Gilabert L[1,2,3], Fernández-García S[1,2,3], Rodriguez-Urgellés E[1,2,3], Soria G[2,4,6,7], Rodríguez MJ[1,2,3], Alberch J[1,2,3], Masana M[1,2,3]

[1] Department of Biomedical Sciences, Institute of Neurosciences, School of Medicine and Health Sciences, University of Barcelona, 08036 Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain
[5] Metofico ltd, London, United Kingdom
[6] Laboratory of Surgical Neuroanatomy, Institute of Neurosciences, School of Medicine and Health Sciences, Universitat de Barcelona, Barcelona, Spain
[7] Consorcio Centro de Investigación Biomédica en Red (CIBER) de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain

Disruption of basal ganglia (BG) circuits underlie several movement disorders, such as Huntington's disease (HD). Recent functional studies reveal that distinct BG sub-circuits modulate different behavioral domains, i.e dorso-medial striatum (DMS) seems more involved in goal-directed action control while dorso-lateral striatum (DLS) in habit and skill learning. Here, we unravel functional implications of DLS and DMS and its main striatal output pathways: direct and indirect (expressing D1 or A2a receptors respectively), in HD pathophysiology.

First, we analyzed functional connectivity alterations of DLS and DMS using rs-fMRI in the R6/1 mouse model of HD. DLS showed reduced functional connectivity with all cortical subregions and thalamus, while DMS connectivity was less affected. Then, we bilaterally injected AAV-DIO-ChR2 in the DLS or DMS of Drd1-Cre and A2A-Cre mouse lines crossed with R6/1 mice. In WT animals, optogenetic stimulation of DLS direct pathway increases locomotion, whereas DLS indirect pathway activation improves motor learning, assessed in an accelerating rotarod. These effects were not observed in symptomatic HD mice, or when DMS selective pathways were stimulated. Then, we expressed the GCaMP6f calcium sensor in Drd1-neurons in the DLS or DMS during the accelerated rotarod task, and recorded fluorescence changes using fiber photometry. We found an abnormal neuronal engagement of the direct pathway in the DLS but not the DMS during all phases of skill learning. We are currently analyzing the contribution of the indirect pathway in this task. Altogether, our data suggests a major involvement of the DLS in HD pathophysiology.

## P.24. REPAIRING DEFECTIVE NEURONAL TRANSLATIONAL CONTROL AMELIORATES BEHAVIORAL PHENOTYPE IN R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

**Castany-Pladevall C**[1,2,3], Garcia-Forn M[1,2,3], Golbano A[1,2,3], Creus-Muncunill J[1,2,3], Fraga U[1,2,3], Marin E[1,2,3], Pérez-Navarro E[1,2,3]

[1] Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain

[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain

Erroneous protein synthesis disrupts cellular fitness causing a variety of disease phenotypes. Neurons are especially sensitive to translational dysregulation owning to the fact that it is essential for synaptic plasticity and neuronal survival. Hence, a precise regulation of translation initiation plays a critical role in learning and memory. Defects in this process have been linked to numerous cognitive disorders including addiction, fragile X syndrome and autism and has also been related to several neurodegenerative disorders like Parkinson's disease.

In this line, our group recently stated that loss of translational control in the striatum leading to an aberrantly increased global cap-dependent protein synthesis is a relevant mechanism in Huntington's disease (HD) pathophysiology. Additionally, its normalization by intraventricular injection of 4EGI-1, an inhibitor of the eIF4E/eIF4G complex assembly, prevented the development of motor deficits in the R6/1 mouse model of HD. In the present work, we show that alterations in translational control extend to other brain regions such as the hippocampus suggesting its involvement, not only in motor deficits, but also in cognitive and memory impairment occurring in HD pathology.

Additionally, in order to explore the potential of normalization of protein synthesis as a therapeutic strategy for HD, we analyzed the effect of a set of repurposed drug candidates, shown to decrease protein synthesis in cellular models and mouse brain.

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## P.25. A SYNTHETIC ANALOGUE OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) IMPROVES MOTOR AND COGNITIVE FUNCTION IN R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

**Solés-Tarrés I**[1], Samperi-Esteve T[1], Alberch J[2,3,4], Vaudry D[5], Xifró X[1]

[1] New Therapeutic Targets Group, Departament de Ciències Mèdiques, Facultat de Medicina. Universitatde Girona, c/ Emili Grahit, nº77, E-17003, Girona, Spain

[2] Departament de Biomedicina, Institut de Neurociències, Facultat de Medicina, Universitat de Barcelona,c/ de Casanova nº143, E-08036, Barcelona, Spain

[3] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[4] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)

[5] Laboratory of Neuronal and Neuroendocrine Communication and Differentiation, Neuropeptides, Neuronal death and Cell plasticity team, Normandie Univ, UNIROUEN, Inserm, 76000 Rouen, France

Huntington's disease (HD) is a neurodegenerative disorder caused by the expression of the mutant huntingtin (mHtt). Motor dysfunction and cognitive impairment are two characteristic symptoms of HDand they are associated to the degeneration of striatum and the dysfunction of hippocampus, respectively. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a multifunctional neuropeptide that acts through three receptors named PAC1R, VPAC1R, and VPAC2R. Recently, wefound PACAP improves cognitive and motor symptoms in HD mice mainly through the PAC1R. Unfortunately, therapeutic use of PACAP is hindered because its poor metabolic stability and becausethe activation of VPAC2R is associated with peripheral side effects. Here, we study the therapeutic potential of the Acetyl- [Ala15, Ala20] PACAP-38-propylamide (PACAP-alg), a PACAP analogue showinghigher affinity to PAC1R and a greater biostability. We found that PACAP-alg (30ug/Kg/day) administrated intranasally for 12 days in R6/1 mice improves motor function evaluated using RotaRod and Balance Beam tests, and cognitive impairment analyzed by T-MAZE test. In the striatum, PACAP-alginduces the increase of number and size of DARPP-32 positive neurons as well as the reduction of mhttaggregates, determined by immunofluorescence. We did not observed changes in striatal volume analyzed by Nissl stain. In the hippocampus, PACAP-alg also reduced the number of mhtt aggregates. Moreover R6/1 mice treated with PACAP-alg showed an increase in number of dendritic spines in CA1 measured by Golgi stain. In conclusion, PACAP-alg administration improves motor and cognitive deficits of R6/1 mice improving neuronal function and enhancing the synaptic plasticity. Thus, the use of this analogue could be considered as a good therapeutic strategy to fight the symptomatology of HD.

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## P.26. ROLE OF CD38 IN TRANSMITOPHAGY FROM ASTROCYTES TO NEURONS IN THE STRIATUM OF HUNTINGTON'S DISEASE

**Pereda-Velarde A**[1]*, López-Molina L[1,2,3]*, Ginés-Padrós S[1,2,3]

[1] Departament de Biomedicina, Facultat de Medicina. Institut de Neurociències. Universitat de Barcelona, Barcelona, Spain.
[2] Institut d'Investigacions Biomèdiques Pi i Sunyer (IDIBAPS), Barcelona, Spain.
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain. *Coauthors

Huntington's disease (HD) is a neurodegenerative disorder characterized by the selective loss of striatal medium spiny neurons. Even though the molecular mechanisms of this striatal vulnerability are still unclear, compelling evidences reveal that mitochondrial dysfunction could be involved. Nevertheless, most of what we know now about this disturbances in mitochondria in HD has been studied only in neurons. Less is known about the regulation of mitochondrial dynamics in glia, specifically in astrocytes. Indeed, it almost has not been studied whether mitochondrial dynamics in astrocytes may be contributing to neuron disturbances and, therefore, to pathology of HD. Thus, we aimed to define the specific contribution of astrocytic mitochondria and the intercellular mitochondria communication between neurons and astrocytes in HD pathology.

For this work, we used the transgenic R6/1 mouse model of HD to either isolate adult striatal astrocytes at different disease stages and cultured primary striatal astrocytes and neurons. First, we studied the intercellular mitochondria communication between astrocytes and neurons. We confirmed the presence of functional mitochondria in the extracellular media by immunofluorescence assays and an internalization of astrocytic mitochondria by striatal neurons. Mitochondria transference induce a toxic effect to neurons, promoting their impairment in HD. This phenomenon could be endorsed due to the activation of the CD38 pathway. Our results demonstrated that there is an increase in CD38 levels in the R6/1 striatum and in human HD's patients caudate. By inhibiting CD38 with siRNA we can prevent astrocytes from releasing aberrant mitochondria to extracellular media. This work provides new insight into how astrocytes could be participating in HD patophysiology. Further studies should contemplate CD38 inhibition to study HD phenotype improvement.

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## P27. INVOLVEMENT OF TRNA FRAGMENTATION IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE

**Guisado-Corcoll A**[1,2,3], Escaramís G[1,4], Brito V[1,2,3], Pérez-Navarro E[1,2,3], Martí E[1,4]

[1] Institut de Neuorciències, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Espanya
[2] Institut d'Investigacions Biomèdiques Agustí Pi i Sunyer (IDIBAPS), Barcelona, Espanya
[3] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Espanya
[4] Centro de Investigación Biomédica en Red sobre Epidemiología y Salud Pública (CIBERESP), Madrid, Espanya

Progressive motor alterations and selective death of medium-sized spiny neurons in the caudate and putamen are key pathological hallmarks of Huntington's disease (HD), a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the coding regions of the huntingtin (HTT) gene. Growing evidence indicates that expanded CAG repeats within mutant HTT mRNA and derived small CAG repeat RNAs (sCAG) participate in HD pathophysiology. However, the role of other classes of small RNAs (sRNA) that are strongly perturbed in HD is uncertain.

Our most recent data indicate that sRNA produced in the putamen of HD patients are sufficient to induce HD pathology in vivo. This observation prompted us to deeply characterize the sRNA transcriptome and identify which sRNA species are enriched in HD putamen and show neurotoxic potential. Specifically, we have observed a massive increase in tRNA fragments (tRFs). tRFs are bioactive molecules that regulate gene expression at multiple levels, whose biogenesis is linked with cellular stress and regulated by post-transcriptional modifications. Our analyses suggest that many of the tRFs over-represented in HD are dependent on the methylation status of the precursor, mature tRNAs. Validating these results, we have detected altered expression of enzymes regulating tRNA post-transcriptional modifications in HD human and mouse brains. We have also studied the potential modifications in the tRNAs, which provides an additional source of pathogenic alterations in HD. These results highlight that multiple sRNA species are contributing to striatal neuropathology, favouring therapeutic strategies based on the blockage of sRNA biogenesis and/or toxic activity.

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## P28. ISOLATION AND CHARACTERIZATION OF HPSC-DERIVED STRIATAL PROGENITOR SUBPOPULATIONS FOR CELL THERAPY IN HUNTINGTON'S DISEASE

**Molina-Ruiz FJ**[1], Sanders P[1], Gomis C[1], Bombau G[1], Galofré M[1], Artigas S[1], Introna C[1], Monforte V[1], Canals JM[1]

[1] Laboratory of stem cells and regenerative medicine, Department of biomedical sciences, and Production and Validation Center of Advanced Therapies (Creatio), Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona; Neuroscience Institute, University of Barcelona, Barcelona; August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona; and Networked Biomedical Research Centre for Neurodegenerative Disorders (CIBERNED).

Huntington's disease (HD) is a currently incurable neurodegenerative disease primarily characterized by the loss of striatal medium spiny neurons (MSNs). Cell replacement therapy (CRT) is the only approach focused on structural and functional restoration of atrophied tissue in HD by replenishing the degenerating MSN population, but its clinical translation is limited by the heterogeneity of cell products. The aim of this work is to develop a novel therapeutic strategy to regenerate the brain tissue affected in HD by using cell sorting to generate safe, defined and reproducible cell products. Here, we describe the identification of a marker for the selection of human pluripotent stem cell (hPSC)-derived striatal progenitors, and a method for the enrichment of these progenitors from heterogeneous cell populations. Furthermore, we characterize this subpopulation to assess its identity and potential to generate MSNs. Finally, we evaluate the survival of these progenitors following transplantation into the striatum of adult mice. We demonstrate that this approach reduces the heterogeneity of the final cell product and batch-to-batch variability using both control and HD hPSC lines. Moreover, we show that different neuroblast subtypes with the potential to generate MSN-like cells can be enriched under different conditions. Finally, we provide evidence of the survival and integration into the striatum of the selected progenitors up to one-week post transplantation. We conclude that the selection of striatal neuroblast populations prior to transplantation has the potential to generate safer and more defined cell products, which can be used to treat HD.

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## P29. COMPLEX IN VITRO MODEL TO STUDY HUNTINTON'S DISEASE

**Introna C**[1], Pereira I[2], López Martínez MJ[2], Tornero Prieto D[1], Samitier J[2], Canals JM[2]

[1] Laboratory of Stem Cells and Regenerative Medicine, Department of Biomedical Sciences, Creatio - Production and Validation Center of Advanced Therapies, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona; IDIBAPS; CIBERNED; Barcelona, Spain.
[2] Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology BIST, Barcelona, Spain

Aim: Huntington's disease (HD) is a severe neurodegenerative characterized by the selective loss of Medium Spiny Neurons (MSNs) within the Caudate/Putamen brain nuclei. These neurons promotes initiation of movements and are highly interconnected with several areas of the brain as the cortex. To better understand how this connection is degraded in HD and enable testing of potential new treatments, we have combined the advantages of microfluidic devices and human pluripotent stem cell (hPSC) differentiation, building a model that allows the in vitro study of the human pathology, by modelling the cortico-striatal circuit affected in HD.

Methods: Our new microfluidic device was built by culturing hPSC-derived cortical cells and MSNs in different compartments interconnected by microchannels for axon isolation.

Results: We showed that cortical and striatal neurons remain isolated in their respective compartments. Furthermore, we demonstrated that the two cultures can be seeded and maintained in parallel with two different differentiation protocols during a time long enough to allow the completion of hPSC differentiation into mature neurons and stablish synaptic connections. Moreover, the device correctly allows MSN differentiation from HD patient-derived hPSCs which survived and were targeted by glutamatergic projections, coming from the cortical compartment and passing through the microfluidic channels.

Conclusions: Our microfluidic devices represent a suitable model for uncovering mechanisms of altered development in HD would allow a better understanding of early phases of the pathology and contribute to find out new therapeutic targets that could be addressed in its very premature stages to prevent the neurodegeneration of MSNs.

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## P.30. CONTRIBUTION OF m$^6$A RNA METHYLATION IN AGE-RELATED COGNITIVE DECLINE AND AD:CHARACTERIZATION IN SAMP8 AND 3xTG-AD MICE MODELS

**Irisarri-Martínez A**[1], Bellver-Sanchis A[1], Companys-Alemany J[1], Pallàs M[1], Griñán-Ferré C[1]

[1]Department of Pharmacology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona, Avda. Joan XXIII, 27. 08028 Barcelona, Spain.

Life expectancy is increasing, and with it, age-related neurodegenerative diseases have become the greatest health-care challenge to this day. Among them, Alzheimer's Disease (AD) is known to be the most common case of dementia. Recently, research on the pathophysiology of AD has focused on the study of epigenetic alterations, more precisely, on N6-methyladenosine (m$^6$A) RNA methylation. m$^6$A has been shown to take part in the development of the nervous system and neural degeneration, and so, it is considered as one of the biological markers regulating gene expression programs in AD. Its methylation is modified by the combined action of various enzymes including METTL3 and FTO.

This study aimed to determine the molecular regulatory mechanism by which m$^6$A methylationlevels contribute to age-related cognitive decline and its interaction with early amyloid deposition. Characterization of m$^6$A in both SAMP8 (6-month-old) and 3xTg-AD (12-month-old) mice models was carried out, respectively. We provided evidence of both an increase in FTO levels as well as a decrease in METTL3 protein levels in SAMP8 mouse model, which was consistent with the group's previous research on human AD brains. In the case of 3xTg-AD model, it seemed to present the opposite tendency. Thus, our results suggest m$^6$A as a novel target for the development of pharmacological drugs.

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## P.31. COGNITIVE AND GABAERGIC PROTECTION IN J20/VLW MICE: A MODEL OF COGNITIVE RESILIENCE TO ALZHEIMER'S DISEASE.

Dávila-Bouziguet E[1,2], Casòliba-Melich A[1,2], Targa-Fabra G[1,2], Galera-López L[3], Ozaita A[3], Maldonado R[3], Ávila J[2,4], Delgado-García JM[5], Gruart A[5], Soriano E[1,2], **Pascual M**[1,2]

[1] Department of Cell Biology, Physiology and Immunology, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain.
[2] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, ISCIII), Spain.
[3] Laboratory of Neuropharmacology-NeuroPhar, Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain.
[4] Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Neurobiology Laboratory, Madrid, Spain.
[5] Division of Neurosciences, Pablo de Olavide University, Seville, Spain.

Alzheimer's disease comprises amyloid-β and hyperphosphorylated Tau accumulation, imbalanced neuronal activity, aberrant oscillatory rhythms, and cognitive deficits. Cognitively resilient Alzheimer's disease (CRAD) defines a novel clinical entity with amyloid-β and Tau pathologies but preserved cognition. The mechanisms underlying such neuroprotection remain undetermined, and animal models of CRAD are currently unavailable.

By neuroanatomical tract-tracing, immunodetection, electrophysiological analyses, and behavioral studies, we characterize a new double transgenic mouse model accumulating amyloid-β and hyperphosphorylated Tau: J20/VLW mice. We demonstrate that J20/VLW animals exhibit preserved hippocampal oscillatory activity and cognition, as opposed to single transgenic J20 and VLW mice, which show significant alterations. Furthermore, we show that the overexpression of mutant human Tau in coexistence with amyloid-β accumulation renders a particular Tau phosphorylation signature in hippocampal interneurons. Moreover, the GABAergic septohippocampal pathway, responsible for hippocampal oscillatory activity, is preserved in J20/VLW mice, in contrast to single mutants.

Our data highlight J20/VLW mice as a suitable animal model in which to explore the mechanisms driving cognitive preservation in cognitively resilient Alzheimer's disease. Moreover, they suggest that a differential Tau phosphorylation pattern in hippocampal interneurons prevents the loss of GABAergic septohippocampal innervation and alterations in local field potentials, thereby avoiding cognitive deficits.

## P.32. REDUCTION IN THE DENSITY OF GIRK CHANNELS AND LOSS OF THEIR CO-CLUSTERING WITH GABAB RECEPTORS IN THE HIPPOCAMPUS OF APP/PS1 MICE

**Martín-Belmonte A**[1], Alfaro-Ruiz R[2], Aguado C[2], Moreno-Martinez AE[2], de la Ossa L[3], Shigemoto R[4], Ciruela F[1], Fukazawa Y[5] and Luján R[2]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, Neuropharmacology & Pain Group, University of Barcelona, L'Hospitalet de Llobregat, Spain.
[2] Synaptic Structure Laboratory, Instituto de Investigación en Discapacidades Neurológicas (IDINE), Dept. Ciencias Médicas, Facultad de Medicina, Universidad Castilla-La Mancha, Campus Biosanitario, C/ Almansa 14, Albacete, Spain.
[3] Departamento de Sistemas Informáticos, Escuela Superior de Ingeniería Informática, Universidad de Castilla-La Mancha, Albacete, Spain.
[4] Institute of Science and Technology (IST Austria), Am Campus 1, A-3400 Klosterneuburg, Austria.
[5] Division of Cell Biology and Neuroscience, Department of Histological and Physiological Sciences, Faculty of Medical Science, University of Fukui, Fukui, Japan.

G protein-gated inwardly rectifying K+ (GIRK) channels are the main targets controlling excitability and synaptic plasticity on hippocampal neurons. Consequently, dysfunction of GIRK-mediated signalling has been implicated in the pathophysiology of Alzheimer´s disease (AD). GIRK channels constitute an important effector component of through GABAB receptors, which have been prove that are involved in AD, to generate slow inhibitory postsynaptic potentials. Using immunoelectron microscopy technique of the highest resolution and sensitivity, the SDS-Freeze fracture Replica Labelling (SDS-FRL) technique, combined with quantitative analyses, we provided for the first time information on how GIRK1 and GIRK2 subunits of the GIRK channels are organised in different compartments of in the stratum radiatum CA1 pyramidal cells of the hippocampus in normal and pathological conditions. The results showed an important reduction in the density of the GIRK1 and GIRK2 along the surface of pyramidal cells in postsynaptic and presynaptic compartments in APP/PS1 mice of 12 months of age. In addition, we also wanted to provide insights into the GABAB–GIRK interaction and how this is altered in transgenic mice using double-labelling SDS-FRL analysing the extent of the spatial relationship between GABAB1 and GIRK2. In wild type mice, immunoparticles for GABAB1 co-clustered with those for GIRK2 predominantly along the extrasynaptic plasma membrane of spines, but also in dendritic shafts and axon terminals. This spatial proximity ensures that signaling is both specific and fast. However, in APP/PS1 mice, we detected that the co-clustering between GABAB receptors and GIRK channels is lost. Overall, our data suggest that the regulation of the signaling mediated through GABAB-GIRK2 is altered, likely contributing to the cognitive dysfunctions associated with AD.

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## P.33. CELL-TYPE HIPPOCAMPAL PATHOLOGY CORRELATES WITH MEMORY DEFICITS IN A NEW ALZHEIMER'S DISEASE MOUSE MODEL

**Deprada A**[1,2], Capilla-López MD[1,2], Avalle S[1], Sanz E[3], Quintana A[3], Parra-Damas A[1,2], Saura CA[1,2]

[1] Departament de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

[2] Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED)

[3] Departament de Biologia Celular, Fisiologia i Immunologia, Institute of Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

Memory deficits in Alzheimer's disease (AD) are associated with excitatory/inhibitory neurotransmission imbalance in memory neural circuits affected by amyloid-β and tau pathologies. However, the specific mechanisms by which these neuropathologies alter excitatory and inhibitory hippocampal neurons remain poorly understood. Here, we report differential gene effects on cognitive performance, as well as selective cell-type pathology in a novel AD mouse model expressing in excitatory neurons familial AD-linked mutant human mutant amyloid precursor protein (APP) and microtubule-associated protein tau (Tau) genes. Additionally, we employ the RiboTag approach to isolate and analyze ribosome binding mRNAs from excitatory and inhibitory neurons in the APP/Tau model. Histopathological analyses reveal that Aβ and hyperphosphorylated Tau are mainly present in excitatory neurons (CaMKIIa+), rather than inhibitory interneurons (Parvalbumin+). At 6 months of age, Tau and APP/Tau mice show spatial learning and memory deficits associated with reduced levels of synaptonuclear factors and synaptic proteins related to excitatory neurotransmission in the hippocampus. Interestingly, tissue clearing and 3D imaging techniques show that the number of inhibitory PV-expressing neurons is selectively reduced in the APP/Tau mice hippocampus, evidencing alterations in inhibitory neurotransmission in our model. To discern the cell type-specific transcriptional programs altered during spatial learning in APP/Tau mice, we generated WT and APP/Tau mice expressing CaMKII-α-Cre;RiboTag and Pvalb-Cre;RiboTag. Using this experimental approach, we isolated and analyzed translating mRNAs of each hippocampal neuronal subpopulation. Globally, our novel APP/Tau;RiboTag mice recapitulate Alzheimer's pathology at the histological, biochemical and behavioral levels, establishing this model as a valuable tool for the study of cell type-specific molecular mechanisms underlying selective neuronal vulnerability in AD.

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## P.34. TARGETING THE MICROGIAL ADP RECEPTOR IN ALZHEIMER´S DISEASE TRANSGENIC MICE

**Giménez I**[1,2], Capilla-López MD[2], Cockram TO J[3], Brown G.C[3], Saura CA[2], Puigdellívol M[1,2]

[1] Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona
[2] Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra
[3] Department of Biochemistry, University of Cambridge, Cambridge, UK

Compelling evidence indicates that microglia play a key role during neurodegeneration, potentially by phagocytosing neurons and synapses and modulating neuronal activity. However, the underlying mechanisms remain unclear. ATP can be released by active and/or stressed synapses and neurons, which induces microglial migration and chemotaxis toward neural injury and synapses via activating the ADP receptor. The activation of this receptor on microglia may lead to an excessive engulfment of synapses and an aberrant modulation of neuronal functions, contributing to synaptic deficits and neuronal dysfunction during neurodegeneration. We show here that pharmacological blockade of the ADP receptor reduces microglial phagocytosis of synapses in vitro. In vivo, we show that chronic pharmacological inhibition of the ADP receptors ameliorates spatial memory and motor coordination dysfunction in a novel APP/Tau mouse, suggesting that blocking the ADP receptor on microglia may be a beneficial treatment for AD.

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## P.35. SOLUBLE EPOXIDE HYDROLASE INHIBITORS COUNTERACT MICROGLIA PHENOTYPIC CHANGES INDUCED BY MONOMERIC C-REACTIVE PROTEIN: NEW PERSPECTIVES AGAINST NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

**Yuan Y**[1], Bartra C[1], Valdés H[1], Martín-López J[2], Mullins JJ[3], Martínez N[2], Codony S[2], García-Lara E[1], Sawkulycz X[4], Morisseau C[5], Hammock BD[5], Vázquez S[2], Muñoz-Torrero D[2], Slevin M[4], Sanfeliu C[1]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona.
[2] Facultat de Farmàcia i Ciències de l'Alimentació, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona.
[3] Le Moyne College, Syracuse, NY.
[4] School of Life Sciences, Manchester Metropolitan University, Manchester.
[5] University of California Davis, Davis, CA.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide. Neuroinflammation is a crucial neuropathological trait in AD, although the underlying mechanisms are not clarified. We previously proposed the monomeric C-reactive protein (mCRP), which is generated by activation and further disaggregation of blood CRP, as a trigger of AD neuropathology after cerebrovascular damage. mCRP is a potent pro-inflammatory agent found deposited in AD brain tissue and proved to induce AD-like dementia and tau and amyloid neuropathology in experimental models. Here we analysed the phenotypic changes induced by mCRP in microglia, the main effector cells of the inflammatory response. Then we tested the protective action of inhibiting the soluble epoxide hydrolase enzyme (sEH), a promising druggable target in AD. sEH inhibitors (sEHi) increase intracellular levels of anti-inflammatory epoxyeicosatrienoic acids (EETs). BV2 microglial cells were incubated with 50 or 100 µM mCRP for 24 h. Leading molecules of chemical families of newly synthesized sEHi (UB-JML-99 and UB-JM-39) were used for protective assays. mCRP activated the nitric oxide pathway as shown by increased release of nitric oxide and higher gene expression of iNOS, whereas sEHi agents blocked these effects. mCRP increased the release of TNFα into the media and the expression of pro-inflammatory cytokines and chemokines. Furthermore, mCRP modified the epigenetic microglial phenotype suggesting a dysregulated pattern. sEHi generally inhibited damaging effects of mCRP with a higher potency than the standard compound TPPU. Overall we demonstrated that mCRP directly activates microglia inflammatory pathways and may contribute to the triggering and progression of AD. Moreover, sEHi were confirmed as effective therapeutic molecules against neuroinflammation in an AD scenario.

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## P.36. PHARMACOLOGICAL INHIBITION OF SOLUBLE EPOXIDE HYDROLASE DURING BRAIN DEVELOPMENT INDUCES LONG-TERM BENEFICES IN 5XFAD MICE

**Villoslada A**[1], Bartra C[1], Irisarri A[1], Corpas R[1], Suñol C[1], Griñán-Ferré C[2], Pallàs M[2], Sanfeliu C[1]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona.
[2] Facultat de Farmàcia i Ciències de l'Alimentació, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona.

Modulation of the risk of Alzheimer's disease (AD) may begin early in life. During embryo development and maturation, the brain receives maternal physiological influences and establishes new epigenetic patterns that build its level of resilience to late diseases. We treated wild-type pregnant mice with the soluble epoxide hydrolase (sEH) inhibitor TPPU until their pups were weaned. The male progenitors were AD transgenic mice from the strain 5XFAD. At two months of age, male and female mice were blindly analyzed for learning and memory performance and their brain tissue was preserved for further analysis. Once genotyped, we found that 5XFAD mice worn from vehicle-treated mothers showed a poor response in cognitive tests of object recognition and spatial location. Notably, those 5XFAD mice from TPPU-treated mothers showed similar performance to their wild-type siblings. At the molecular level, tau pathology shown by increased hippocampal p-tau levels in 5XFAD mice was totally prevented in those whose mothers were treated with TPPU. Furthermore, TPPU treatment also modified the expression of epigenetic markers. Overall, we confirmed the potential of the enzyme sEH as a new target to fight AD and demonstrated that its inhibition in the developing brain produces long-term preventive effects against neurodegeneration.

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## P.37. THE Δ$^9$-TETRAHYDROCANNABINOL AND CANNABIDIOL COMBINATION REDUCES THE EXCESSIVE GLUTAMATERGIC ACTIVITY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

**Sánchez-Fernández** N[1], Gómez-Acero L[1], Castañé A[2,3], Campa L[2], Brito V[4], Ginés S[4], Ciruela F[1], Aso E[1]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, IDIBELL, L'Hospitalet de Llobregat, Spain
[2] Institut d'Investigacions Biomèdiques de Barcelona (IIBB-CSIC), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[3] Facultat de Medicina, Universitat de Vic-Universitat Central de Catalunya (UVic-UCC), Vic, Spain
[4] Departament de Biomedicina, Facultat de Medicina, Institut de Neurociències, Universitat de Barcelona; Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Alzheimer's disease (AD) is the most common form of dementia and is characterized by a progressive loss of memory and other mental abilities. Current therapies against AD are not totally effective, which highlights the need for new therapeutic strategies. Previous results from our group demonstrated that a combination of non-psychoactive doses of the natural cannabinoids Δ$^9$-tetrahydrocannabinol (THC) and cannabidiol (CBD), the main components of the first cannabis-based medicine approved in many countries, reduces cognitive decline in a mouse model of AD, the APP/PS1 mice. However, the molecular mechanisms underlying this therapeutic effect are not completely understood. Here, we studied the effects of THC and CBD on the glutamate homeostasis and on synaptic plasticity in the hippocampus of APP/PS1 mice because these two processes are known to be altered in AD. Thus, by using *in vivo* microdialysis and HPLC techniques, we have quantified the glutamate levels in the hippocampus of wild-type and APP/PS1 animals in response to veratridine and the glutamate transporter-1 inhibitor dihydrokainate (DHK) after a chronic treatment with THC and/or CBD.

Interestingly, THC+CBD treatment reduced the veratridine-evoked glutamate release in both genotypes and attenuated the enhanced glutamate levels observed in DHK-treated APP/PS1 mice. In contrast, our results by using ballistic labelling demonstrated that THC+CBD chronic treatment does not significantly impact on dendritic spine density and morphology in the hippocampus of APP/PS1 mice. These results suggest that cognitive improvement after THC+CBD treatment could be related with a reduction of the excitotoxicity occurring in our AD model.

## P.38.THE LACK OF GADD45 PROTEIN IN MICE LEADS TO UNEXPECTED COGNITIVE IMPAIRMENT AND ADHALLMARKS

**Jarne-Ferrer J**[1], Griñán-Ferré C[1], Jurado-Aguilar J[2], Barroso E[2,3], Salvador J[4], Palomer X[2,3], Vázquez-Carrera M[2,3], Pallàs M[1]

[1] Department of Pharmacology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona, Avda.Joan XXIII. 08028 Barcelona, Spain

[2] Spanish Biomedical Research Center in Diabetes and Associated Metabolic Diseases (CIBERDEM)-Instituto de Salud Carlos III, Madrid, Spain

[3] Pediatric Research Institute-Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain; Departament of Immunology & Oncology lab 417 National Center for Biotechnology, CSIC, C/Darwin, Campus Cantoblanco. 28049 Madrid, Spain.

Growth arrest DNA damage-inducible protein (GADD45) is implicated in different responses to cell injury, suggesting that this protein may participate in survival mechanisms, including apoptosis, cell cycle arrest or DNA repair. Furthermore, these cell processes are particularly relevant in metabolism, immunity or longevity, among others. Therefore, GADD45 protein is related to aging and the control of life span, suggesting that it could be a potential therapeutic target in age-related diseases such as Alzheimer Disease (AD).

In this study, we used Wild Type (WT, n=15) and GADD45 Knock Out (KO, n=15) mice models toevaluate the role of this protein in AD progression. Since it has been shown that insulin resistance (IR) could be involve in the development of cognitive impairment, glucose tolerance test was performed to determine if the lack of this protein caused IR at 4-month-old mice. Although no differences were found, behavioral tests were performed to evaluate mice cognitive state as well as molecular analysis of AD through WB and qPCR techniques. Behavioraltests showed that GADD45 KO mice presented cognitive deficits compared to WT animals. Molecularly, these GADD45 KO animals showed significantly increased levels of proinflammatory cytokines and tau pathology features in the brain. These findings demonstrated that the lack of GADD45 protein exacerbates AD pathology, which suggest that this protein might be a potential target to slow down AD progression.

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## P.39. CB2R-OX1R HETEROMER AS A NEW THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE

**Rebassa JB**[1], Raïch I[1], Contestí J[1], Lillo A[1,3], Lillo J[2,3], Franco R[2,3], Navarro G[1,3]

[1] Facultat de Farmàcia, Departament de Bioquímica i i Fisiologia, Universitat de Barcelona, Barcelona
[2] Facultat de Biologia, Departament de Bioquimica i Biologia Molecular, Universitat de Barcelona, Barcelona
[3] CIBERNED

CB1 and CB2 cannabinoid receptors are being considered as possible key components to decrease the progression of Alzheimer's disease. Both proteins are G protein coupled receptors (GPCR). On the one hand, CB1R is the most abundant receptor in the Central Nervous System (CNS), provoking the psychoactive effects induced by cannabinoid compounds. On the other hand, CB2R is principally located in the cells of the immune system, even though recently it has been demonstrated its presence on the CNS.

It is well accepted that CB2R shows neurological protective benefits in the neuroinflammation conditions present in Alzheimer disease where the receptor expression is increased. Different data describe that CB2R can be regulated by the action of OX1 receptor. Orexin is a hormone that acts as a neuropeptide in the Central Nervous System. Thus, the main objective of our study consists in analysing the possible interaction between cannabinoid CB2 ant orexin OX1 receptors and characterize the functionality of the CB1-OX1 receptor heterodimer. In this sense, it has been demonstrated that CB2 and OX1 receptors are expressed and colocalize at the plasma membrane. In addition, it has been shown that they can interact forming an heteromeric complex CB2R-OX1R in HEK-293T co-transfected cells and microglial and neuronal primary cultures. Moreover, a proximity ligation assay (PLA) has been carried out obtaining that Aß induce an important increase of the heteromer expression.

To elucidate the signalling pathway, it has been observed that OX1R inhibition potentiates the CB2R functionality in transfected HEK-293T cells and primary cortical neurons. Moreover, it has been demonstrated that the TM4-TM5 are involved in the formation of the heteromeric CB2R-OX1R complex. In conclusion, the CB2R-OX1R complex could be used as a new therapeutic target to treat Alzheimer disease.

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## P.40. TREATMENT MODULATION OF SERUM LEVELS OF NEURAL-PLASTICITY RELATED miRNAS IN A SPORADIC RODENT MODEL OF ALZHEIMER'S DISEASE

**Riberas A**[1], Puig-Parnau I[1], Vila-Soles L[2], Garcia-Brito S[2], Gubern C[3], Aldavert-Vera L[2], Segura-Torres P[2], Huguet G[1], Kádár E[1]

[1] *Departament de Biologia, Universitat de Girona, Girona, Spain*
[2] *Unitat Psicobiologia, Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain*
[3] *Cerebrovascular Pathology Research Group, Department of Neurology, Girona Biomedical Research Institute (IDIBGI), Girona, Spain*

Deep brain stimulation (DBS) has been suggested as a novel treatment for Alzheimer's disease (AD). Previous research demonstrates that Intracranial self-stimulation (ICSS) applied to medial forebrain bundle (MFB) facilitates learning and memory. At the molecular level, ICSS-MFB regulates the expression of specific miRNAs related to synaptic plasticity and postulated as potential biomarkers of AD progression.

This study approaches the effects of ICSS-MFB on serum expression levels of miRNAs in a sporadic rodent AD model. Rats received 2 mg/Kg of streptozotocin (STZ) or citrate buffer. ICSS treatment (5 sessions of 45 minutes) was administered contingent with Morris Water Maze acquisition (A) phase on days 33 to 37 post-STZ. Hippocampal levels of pTau S202/T205, APP and Sirt-1 proteins or miRNAs were analysed by western blot or qRT-PCR to confirm molecular hallmarks of pathology in STZ vs control rats at 40 days post-STZ. Serum levels of miRNAs were quantified by qRT-PCR in samples obtained immediately after the last ICSS or Sham session.

Hippocampal levels of pTau, APP, Sirt-1 and miR-let-7b-5p were significantly increased in AD rats, which also showed increased latencies in A3 session compared to controls. No significant differences were observed in serum expression of the analysed miRNAs, except for miR-181c. AD rats displayed significantly lower levels of miR-181c, but administration of ICSS-MFB resulted in similar miR-181c levels to control rats. ICSS-MFB treated AD rats also showed significant lower latencies in A3 and A5 sessions compared to non-treated AD rats. A negative correlation was observed between miR-181c levels and latency in A5 session, mean distance to target during the first 60 seconds and miR-181a levels.

Our results indicate that ICSS-MFB restores miRNA-181c serum levels in AD-like conditions and lend support to the promising potential of specific miRNAs to measure the effectiveness of DBS treatments to combat AD cognitive deficits.

## P.41. LIPIDOMICS ALTERATIONS OF WHITE MATTER IN ALZHEIMER'S DISEASE PATHOLOGY

**Obis E**[1], Sol J[1], Andres-Benito P[2], Martín-Gari M[1], Mota-Martorell N[1], Galo-Licona JD[1], Portero-Otin M[1], Ferrer I[2,3], Pamplona R[1], Jové M[1]

[1] Department of Experimental Medicine, University of Lleida-Lleida Biomedical Research Institute (UdL-IRBLleida), 25198, Lleida, Spain.
[2] Center for Biomedical Research On Neurodegenerative Diseases (CIBERNED), Institute of Health Carlos III, 28220, Madrid, Spain.
[3] Department of Pathology and Experimental Therapeutics, University of Barcelona, L'Hospitalet de Llobregat, 08907, Barcelona, Spain.

The main objective of this study was to increase our understanding of the role of brain lipids in pathophysiology of aging and Alzheimer's disease (AD) with a special focus on the alterations of the white matter. To achieve this objective, we undertook a non-targeted lipidomics analysis of post-mortem frontal cortex area 8 grey matter, and subjacent white matter (WM) to define lipidomic changes and potential biomarkers that distinguish healthy subjects from those with AD and are representative of AD progression. Lipidomic analyses using a LC-MS/MS platform were carried out in the frontal WM and grey matter of AD cases at different Braak and Braak stages (n=6 healthy subjects; n=6 AD cases Braak and Braak I-II/0-A; n=6 AD cases Braak and Braak III-IV/0-C; and n=6 AD cases Braak and Braak V-VI/B-C) without clinical or pathological co-morbidities to analyze lipid patterns. This election is based on the fact that most individuals with AD neuropathological changes have co-morbidities which have an impact on the integrity of the WM.

Lipidomics analyses showed significant differences between WM and grey matter in healthy subjects ascribed to plasmalogens, ceramides, and glycosphingolipids and a specific fatty acid profile affecting preferentially to 16:0, 18:1 and 22:6, suggesting region-specific differences in fatty acid synthase, SCD1 and peroxisomal beta oxidation. Notably, changes detected in AD affect preferentially the lipids species and fatty acids that differentiate both regions, and that change by increasing or decreasing with AD progression. Based on these results we conclude that there are specific lipidomic profiles that differentiates WM from grey matter in AD and also to define AD progression. These lipidomics alteration point to alterations in fatty acid metabolism, as well as glycerophospholipids and sphingolipid biosynthesis pathways.

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## NEUROTRANSMITTER RECEPTORS AND NEUROPHARMACOLOGY

### P.42. IDENTIFICATION OF CB2-GHS-R1A HETEROMER FUNCTIONALITY AND MARKED UPREGULATION IN STRIATAL NEURONS FROM OFFSPRING OF MICE UNDER A HIGH-FAT DIET

**Lillo J**[1,2], Lillo A[3], Zafra DA[3], Miralpeix C[4], Rivas-Santisteban R[1,2], Casals N[4,5], Navarro G[1,3]*, Franco R[1,2]*

[1] Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, C/Sinesio Delgado, 4, 28029 Madrid, Spain.
[2] Molecular Neurobiology Laboratory. Department of Biochemistry and Molecular Biomedicine.
[3] University of Barcelona, Diagonal 643, 08028 Barcelona, Spain.Department of Biochemistry and Physiology. School of Pharmacy. University of Barcelona, 08028 Barcelona. Spain.
[4] Basic Sciences Department, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain

Cannabinoids have been reported as orexigenic, i.e. as promoting food intake that, among others, is controlled by the so-called “hunger” hormone, ghrelin. The aim of this project focus in looking for functional and/or molecular interactions between ghrelin GHS-R1a and cannabinoid CB2 receptors at the central nervous system (CNS) level. In a heterologous system we identified CB2-GHS-R1a receptor complexes with a particular heteromer print consisting of impairment blockade of CB2 receptor/Gi-mediated signaling. The blockade was due to allosteric interactions within the heteromeric complex as it was reverted by antagonists of the GHS-R1a receptor. On the other hand, cannabinoids acting on the CB2 receptor did not affect cytosolic increases of calcium ion induced by ghrelin acting on the GHS-R1a receptor. Finally, in situ proximity ligation imaging assays confirmed the expression of CB2-GHS-R1a receptor complexes in both heterologous cells and primary striatal neurons. Interestingly, there was a marked upregulation of those complexes in striatal neurons from siblings of pregnant female mice under a high-fat diet.

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## P.43. COMPLEX PHENOTYPE OF A "DE NOVO" GRIA1 MUTATION ASSOCIATED WITH DEVELOPMENTALENCEPHALOPATHY

**Tubio I**[1], Castellanos A[1], Altafaj X[1,2], Soto D[1,2]

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute ofNeurosciences, Universitat de Barcelona, 08036 Barcelona, Spain

[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain

For the correct brain function, neuronal networks must present functional integrity, which is accomplished by the correct synaptic transmission course. The majority of the synapses in the central nervous system use glutamate as neurotransmitter, which activates a variety of receptors. There are three main types of ligand-gated glutamate receptors (NMDAR, AMPAR and Kainate receptors), which serve different functions in neurons. AMPA receptors (AMPARs) are responsible for approximately the 90% of fast-excitatory neurotransmission and their responses depends on several factors. Importantly, the subunit composition of the receptor (GluA1 to GluA4) determines the kinetic responses upon glutamate binding or single-channel conductance. Any genetic alteration that translates into an amino acid change in the pore-forming subunits will interfere with the normal AMPAR functioning and might have an important consequence on the correct function of the neuronal network. That might be the casefor a paediatric patient with intellectual disability carrying a missense variation in the subunit GluA1 (codified by GRIA1 gene) that translates into an Alanine to Threonine change in the position 636. To determine the impact of this change on GluA1 gating, we have studied in heterologous systems AMPAR- mediated currents in outside-out patches by rapid application of glutamate and we have compared wild-type homomeric AMPARs formed by GluA1 subunits with homomeric GluA1(A636T). In the presence of the agonist, mutant AMPARs exhibit a slower desensitization rate, together with higher steady-state current, indicating an altered receptor kinetics resulting on a prolonged open state configuration. In contrast to this gain of function, our preliminary data also indicate a reduced channel conductance of GluA1(A636T)-containing AMPARs, suggesting a loss-of-function component. Overall, these findings indicate that GluA1(A636T) variant provokes a complex phenotype to GluA1 homomeric receptors disturbing AMPAR activity and probably underlying AMPAR-mediated signalling in the clinical case.

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## P.44. FUNCTIONAL ANNOTATION OF DE NOVO GRIN2A VARIANTS OF THE NMDA RECEPTOR

**Peña-Duclaud X**[1], García-Díaz R[1], Castellanos A[1], Picañol X[1], Olivella M[2], Soto D[1,3], Altafaj X[1,3]

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute ofNeurosciences, Universitat de Barcelona, Barcelona, Spain.
[2] Bioinfomatics and Medical Statistics Group, University of Vic-Central University of Catalonia, Vic, Spain.
[3] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

The glutamatergic neurotransmission mediates the majority of central nervous system excitatory pathways. Several ionotropic receptors with distinct roles (AMPA, NMDA and KA receptors) are expressed at glutamatergic synapses. While AMPARs serve the depolarization needed for neuron-to- neuron communication, NMDARs are the primary inductors of synaptic plasticity processes. Upon their activation, NMDARs allow Ca2+ influx through the channel pore into the neuron, starting the synaptic plasticity changes that constitute the cellular mechanism of learning and memory. When NMDARs do not gate properly, calcium signaling is altered and plasticity might be compromised. One of the causes for primary NMDAR dysregulation is the presence of a de novo mutation affecting GRIN genes, which encode for the GluN subunits. During the last years, there has been an increasing number of reported GRIN variants in patients with neurodevelopmental disorders that has led to the categorization of these neurodevelopmental alterations under the term GRINpathies. The determination of the functional impact of these alterations in the context of GRINpathies is important for future personalized treatments and/or predictions based on the annotation outcome. In this framework, we have examined the effect of GRIN2A(p.V563L) and GRIN2A(p.G664S) on NMDAR-mediated currents by means of electrophysiological recordings on cells transfected with heteromeric GluN1-GluN2A, GluN1-GluN2A(V563L) or GluN1- GluN2A(G664S) NMDARs. While the V563L change translates into a minor hypofunction with NMDAR- mediated currents very similar to wild type receptors, the impact of G664S in the GluN2A subunit correlates with a significant decrease of NMDAR-mediated currents. Our results are in accordance with the location of the mutations and modelling predictions: the more severe mutation G664S is located in the linker between the ligand binding domain and the pore entrance while the milder V563L locates at the external part of transmembrane 1, which is not involved in the formation of the pore.

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## P.45. NUTRACEUTICAL COMPOUND POTENTIATES THE NMDA RECEPTOR

**Picañol X**[1], Altafaj X[1,2], Castellanos A[1], Soto D[1,2], Olivella M[3]

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute ofNeurosciences, Universitat de Barcelona, 08036 Barcelona, Spain.
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain.
[3]Bioinfomatics and Medical Statistics Group, University of Vic-Central University of Catalonia, 08500 Vic, Spain.

Glutamatergic neurotransmission plays a central role in the nervous system, with an impact in key processes such as learning and memory. In this context, the N-methyl-D-aspartate receptor (NMDARs) isan essential ion channel in glutamatergic synapses and recent developments have led to the identification of de novo genetic variants affecting GRIN genes – which encode for NMDAR subunits – associated with neurodevelopmental disorders called GRIN-related disorders (GRDs). These pathogenic variants can provoke different functional phenotypes to the receptor producing either a gain- or loss-of-function (GOF and LOF, respectively). Some evidence has demonstrated that NMDAR hypofunctionality could be pharmacologically rescued. For instance, our group showed that L-serine dietary supplementation results on a clinical improvement in a pediatric patient carrying a LOF GRIN variant.Nevertheless, new therapeutic compounds with higher specificity and/or potency are still necessary. In this context, we aimed to evaluate a novel positive allosteric modulator of the NMDAR, towards the functional rescue of LOF GRIN variants associated with GRDs.

In this work, based on computational studies, we identified a candidate natural compound with a potential positive allosteric modulatory effect on GluN2B subunit-containing NMDARs. In order to functionally evaluate this hypothesis, electrophysiological whole-cell recordings in NMDAR-expressing HEK-293T cells have been conducted. These functional studies showed that the candidate molecule potentiates around 2-fold the current amplitude in (GluN1)2-(GluN2B)2-expressing cells. This effect was not detected in GluN2A-containing NMDARs. In conclusion, our data suggest that this compound can beused as a specific potentiator for the treatment of GRIN2B-related disorders.

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## P.46. EVALUATION OF EAR-20 PEPTIDE AS A POSITIVE ALLOSTERIC MODULATION OF NMDA RECEPTORS

**García-Diaz R**[1,2,3], Castellanos A1, Vega-Castro N[2], Gasull X[1], Reyes-Montaño EA[2] & Soto D[1]

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute of Neurosciences, Universitat de Barcelona, 08036 Barcelona, Spain. Institut d'Investigacions BiomèdiquesAugust Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain.
[2] Grupo de Investigación en Proteínas, Departamento de Química, Universidad Nacional de Colombia, Cra30 No 45-03 Edificio 451 Laboratorio 201-1, Bogotá, Colombia.
[3] Grupo de Investigación en Química y Biotecnología (QUIBIO), Universidad de Santiago de Cali, Santiago de Cali 760035, Colombia

Glutamate is an amino acid that plays an important role in energy metabolism and is the main excitatory neurotransmitter in the central nervous system. It acts on multiple glutamate receptors, including N- methyl-D-aspartate receptors (NMDARs), an important subtype involved in rapid excitatory synaptic transmission. Furthermore, the stimulation of NMDARs allows a Ca2+ influx through the ion channel, regulating synaptic plasticity, and key cellular mechanism involved in learning and memory. Evidence from experimental, clinical and genetic studies implicates NMDAR hypofunction in schizophrenia, intellectual disability, autism and Alzheimer's disease. These conditions have stimulated the interest for positive allosteric modulators (PAMs) of NMDARs as a potential therapeutic strategy for treating the associated cognitive deficits. Based on structural analysis between the GluN2B subunit and conantokin- G, a toxin that interacts selectively with the GluN2B subunit, we have recently designed various peptides(EAR-16, EAR-18 and EAR-20) that are predicted to act on NMDARs. In this work we describe the evaluation of EAR-20 peptide as a new class of positive allosteric modulator of NMDARs. We have tested the effect of EAR-20 by means of electrophysiological recordings on HEK293T cells transfected with heteromeric GluN1-GluN2A or GluN1-GluN2B NMDARs. We found that EAR-20 potentiates whole-cell NMDAR-mediated currents. Although EAR-20 has a similar effect in both GluN2A and GluN2B heteromeric receptors, however, the modulating effect on triheteromeric NMDARs containing GluN2A and GluN2B is significantly lower. Besides, EAR-20 peptide is able to partially activateNMDARs even in the total absence of natural co-agonists (glutamate and glycine). Our results demonstrate that rational design of peptides is a good strategy to get new potential therapeutic agents for schizophrenia and other loss-of-function related disorders.

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## P.47. VARIABLE MODULATION OF AMPA RECEPTORS BY THE AUXILIARY SUBUNITS TARPS

**Castellanos A**[1], Picañol X[1], Miguez-Cabello F[1], Soto D[1,2]

[1] Neurophysiology Laboratory, Physiology Unit, Department of Biomedicine, Medical School, Institute ofNeurosciences, Universitat de Barcelona, 08036 Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain

AMPA receptors (AMPARs) are fundamental components of fast excitatory neurotransmission and are necessary for neuronal plasticity. Their functional properties in neurons are crucially controlled by TARPs(Transmembrane AMPAR Regulatory Proteins), which are highly abundant in the CNS. TARPs are divided in three subfamilies: type Ia (g-2 and g-3), type Ib (g-4 and -8) and type II (g-5 and g-7). All TARPs importantly affect biophysical properties such as gating and pharmacology of AMPARs. However, they show differences in the fine AMPAR modulation. All these differences in AMPAR fine tuning are important in determining the specific behaviour of the postsynaptic neuron in the process of neuron-to- neuron communication. We have previously reported how the prototypical TARP 2 differentially modulates biophysical properties of the channel depending on a given AMPAR:TARP stoichiometry. However, whether this modulation is shared by other members of the family is an open question. Thus, we have tested the stoichiometrically-dependent modulatory effect on representative members for each subfamily: g-2, g-4 and g-5. By means of electrophysiological recordings in transiently transfected HEK293T cells we have studied the effect of a variable number of TARPs in the AMPAR structure. Our data revealed important differences between subfamilies in terms of AMPAR modulation. In contrast to g-2, g-4 and g-5 enhance single-channel conductance with less TARPs into the complex. Other modulatory effects (desensitization kinetics or recovery from desensitization) also differ. Interestingly, some fully TARPed conditions are not favoured as we observed very small currents indicating that a 4-TARPed AMPARs might not be present in neurons. Due to the great variability of TARPs and their differential expression between neuronal subpopulations,these results will help us to better understand the complexity in the regulation of AMPARs and to avoid side effects derived from the use of drugs targeting AMPARs, which effect is dependent on the TARP associated.

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## P.48. BIASED G PROTEIN-INDEPENDENT SIGNALING OF STRIATAL DOPAMINE D1-D3 RECEPTOR HETEROMERS IN RESERPINIZED MICE

**Moreno E**[1,2], Guitart X[3], Rea W[3], Llopart, N[1,2], Cortés A[1,2], Canela E.I[1,2], Ferré S[3]*, Casadó V[1,2]*

[1] Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain.
[2] Institute of Biomedicine of the University of Barcelona (IBUB).
3 Integrative Neurobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, United States.
*Co-senior authors

Several studies found in vitro evidence for heteromerization of dopamine D1 receptors (D1R) and D3 receptors (D3R), and it has been postulated that functional D1R-D3R heteromers that are normally present in the ventral striatum mediate synergistic locomotor-activating effects of D1R and D3R agonists in rodents. Based also on results obtained in vitro, with mammalian transfected cells, it has been hypothesized that those behavioral effects depend on a D1R-D3R heteromer-mediated G protein-independent signaling. Here, we demonstrate the presence on D1R-D3R heteromers in the mouse ventral striatum by using a synthetic peptide that selectively destabilizes D1R-D3R heteromers. Parallel locomotor activity and ex vivo experiments in reserpinized mice and in vitro experiments in D1R-D3R mammalian transfected cells were performed to dissect the signaling mechanisms of D1R-D3R heteromers. Co-administration of D1R and D3R agonists in reserpinized mice produced synergistic locomotor activation and a selective synergistic AKT phosphorylation in the most ventromedial region of the striatum, in the shell of the nucleus accumbens. Application of the destabilizing peptide in transfected cells and in the shell of the nucleus accumbens allowed demonstrating that, both in vitro and in vivo, co-activation of D3R induces a switch from G protein-dependent to G protein-independent D1R-mediated signaling determined by D1R-D3R heteromerization. The results therefore demonstrate that a biased G protein-independent signaling of D1R-D3R heteromers localized in the shell of the nucleus accumbens mediate the locomotor synergistic effects of D1R and D3R agonists in reserpinized mice.

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## P.49. ADENOSINE A2A AND A3 RECEPTORS ARE ABLE TO INTERACT WITH EACH OTHER. FUNCTIONAL CHARACTERIZATION OF THE NEW DESCRIBED HETEROMER AND EXPRESSION LEVELS IN DIFFERENT BRAIN REGIONS

**Lillo A**[1,2], Raïch I[1,2], Lillo J[2,3], Reyes-Resina I[1,2], Navarro G[1,2], Franco F2,3

[1] Department of Biochemistry and Physiology. School of Pharmacy. University of Barcelona, 08028 Barcelona. Spain
[2] Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, C/Sinesio Delgado, 4, 28029 Madrid, Spain
[3] Molecular Neurobiology Laboratory. Department of Biochemistry and Molecular Biomedicine. University of Barcelona, Diagonal 643, 08028 Barcelona, Spain

The aim of this study was to check the possible interaction of two of the four purinergic P1 receptors, the A2A and the A3. Discovery of the A2A–A3 receptor complex was achieved by means of immunocytochemistry and of bioluminescence resonance energy transfer (BRET). The functional properties and heteromer print identification were addressed by signaling assays such as cAMP accumulation and ERK1/2 phosphorylation. The physiological role of the novel heteromer is to provide a differential signaling depending on the pre-coupling to signal transduction components and/or on the concentration of the endogenous agonist. The main feature was that the heteromeric context led to a marked decrease of the signaling originating at A3 receptors. Interestingly from a therapeutic point of view, A2A receptor antagonists overrode the blockade, thus allowing A3 receptor-mediated signaling. In in situ proximity ligation assays (PLA) performed in primary cells, A2AA3Het expression was markedly higher in striatal than in cortical and hippocampal neurons, whereas it was similar in resting and activated microglia. Finally, the expression of the heteromer was markedly enhanced in microglia from the APPSw,Ind model of Alzheimer's disease.

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## P.50. ENVIRONMENTAL LEVELS OF CARBARYL IMPAIR ZEBRAFISH LARVAE BEHAVIOUR: THE POTENTIAL ROLE OF ADRA2B AND HTR2B

Faria M[1], Bellot M[2], **Bedrossiantz J**[1] , Rosas Ramírez R J[3] , Prats E4 , Garcia-Reyero N[5] , Gomez-Canela C[2] , Mestres J[6] , Rovira X[7] , Barata C[1] , Manuel Gómez Oliván L[3], Llebaria A[7] , Raldua D[1]

[1] Institute for Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona, 18, 08034 Barcelona, Spain
[2] Department of Analytical Chemistry and Applied (Chromatography section), School of Engineering, Institut Químic de SarriÀ-Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
[3] Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad AutÓnoma del Estado de MÉxico, Paseo ColÓn intersecciÓn Paseo Tollocan s/n. Col. Residencial ColÓn, 50120 Toluca, Estado de MÉxico, Mexico
[4] Research and Development Center (CID-CSIC), Jordi Girona 18, 08034 Barcelona, Spain
[5] Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS, USA
[6] Chemotargets, IMIM-Hospital del Mar, Universitat Pompeu Fabra, Barcelona, Spain
[7] MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), 08034 Barcelona, Spain

The insecticide carbaryl is commonly found in indirectly exposed freshwater ecosystems at low concentrations considered safe for fish communities. In this study, we showed that after only 24 h of exposure to environmental concentrations of carbaryl (0.066–660 ng/L), zebrafish larvae exhibit impairments in essential behaviours. Interestingly, the observed behavioural effects induced by carbaryl were acetylcholinesterase-independent. To elucidate the molecular initiating event that resulted in the observed behavioural effects, in silico predictions were followed by in vitro validation. We identified two target proteins that potentially interacted with carbaryl, the α2B adrenoceptor (ADRA2B) and the serotonin 2B receptor (HTR2B). Using a pharmacological approach, we then tested the hypothesis that carbaryl had antagonistic interactions with both receptors. Similar to yohimbine and SB204741, which are prototypic antagonists of ADRA2B and HTR2B, respectively, carbaryl increased the heart rate of zebrafish larvae. When we compared the behavioural effects of a 24-h exposure to these pharmacological antagonists with those of carbaryl, a high degree of similarity was found. These results strongly suggest that antagonism of both ADRA2B and HTR2B is the molecular initiating event that leads to adverse outcomes in zebrafish larvae that have undergone 24 h of exposure to environmentally relevant levels of carbaryl.

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## P.51. THE CELL CYCLE REGULATOR CYCLIN D1 MODULATES THE ACTIVITY OF α4-CONTAINING GABAA RECEPTORS

**Pedraza N**[1], Monserrat MV[1], Ferrezuelo F[1], Torres J[1], Colomina N[1], Soto D[2], Miguez F[2], Esteban JA[3], Egea J[4], Garí E[1]

[1] Cell Cycle, Department of Basic Medical Sciences, Institut de Recerca Biomèdica de Lleida (IRBLLEIDA), University of Lleida, Lleida, Spain.
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain. Laboratori de Neurofisiologia, Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain.
[3] Department of Molecular Neurobiology, Centro de Biología Molecular 'Severo Ochoa', Consejo Superior de Investigaciones Científicas (CSIC), Spain.
[4] Molecular and Developmental Neurobiology, Dept. Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida/IRBLLEIDA, Rovira Roure 80, 25198, Lleida, Spain.

The function of the G1 cyclin D1 (Ccnd1) and its catalytic enzymes cyclin-dependent kinase 4 and 6 (Cdk4/6) in cell cycle regulation is well-known, but non-canonical roles of Ccnd1 both in the nucleus and in the cytoplasm are also well established. Some previous studies support a role for Ccnd1 in the nervous system since Ccnd1-null mice show neurological abnormalities and nuclear Ccnd1 promotes proliferation of neuronal precursors, moving to the cytoplasm at the onset of differentiation. However, a cytoplasmic function for Ccnd1 in neurons has not been considered. Here we performed a yeast two-hybrid screening to search for new Ccnd1 interactors in the brain, and found that Ccnd1 interacts with the α4 subunit of gamma-aminobutyric acid (GABA) type A receptors (GABAARs). This interaction has been validated in HEK293 cells and in hippocampal neurons. Moreover, the Ccnd1·Cdk4 complex phosphorylates the α4 subunit at threonine 423 and serine 431, which has functional consequences on GABAARs increasing their surface levels and decreasing their rundown in whole-cell patch-clamp recordings. In accordance, inhibition of Cdk4 by Palbociclib decreases tonic currents and miniature inhibitory postsynaptic current amplitude in the hippocampus of newborn rats. Our findings suggest the involvement of a cyclin in neuronal signaling and highlight a novel cytoplasmic function of Ccnd1·Cdk4 in the regulation of GABAAR efficacy in the central nervous system.

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## DISEASE BIOMARKERS, MECHANISMS AND THERAPIES

### P52. BIOINFORMATIC TOOLS TOWARDS GRIN VARIANTS STRATIFICATION

**Olivella M**[1]*, Santos-Gómez A[2,3], García-Recio A[1], Miguez-Cabello F[2,3], Soto D[2,3], Altafaj X[2, 3]*

[1] Bioinfomatics and Medical Statistics Group, University of Vic - Central University of Catalonia, 08500 Vic

[2] Neurophysiology Laboratory, Department of Biomedicine, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, 08036 Barcelona

[3] August Pi i Sunyer Biomedical Research Institute (IDIBAPS), 08036 Barcelona

The existence of autosomal dominant de novo GRIN gene variants associated with GRIN-related disorders (GRDs), a rare pediatric neurological disorder caused by N-methyl- d-aspartate receptor (NMDAR) dysfunction, has been recently identified. GRIN variants identification is exponentially growing and in order to design precision therapeutic strategies it is essential to functionally stratify variants, both in terms of pathogenicity (neutral vs. disease-associated variants) and functional impact (loss-of-function, neutral, gain-of-function). Unfortunately, to date GRIN variants clinical, genetic, and functional annotations remain highly fragmented, which constitutes a bottleneck in GRDpatient's stratification. To address this issue, we have developed GRIN database (https://alf06.uab.es/grindb/home), a publicly available, non-redundant, updated, and manually-curated database containing all available genetic, functional, and clinical data from more than 4000 GRIN variants reported worldwide. The analysis of GRIN database shows that approximately 50% of disease-causing GRIN variants still lack a functional annotation, and thus can not be stratified to further undergo personalised therapies. To overcome this problem, we have thus developed a structure-based computational algorithm with the aim of predicting the pathogenesis and the functional annotations of non-annotated GRIN variants. The algorithm is based on the hypothesis that functional annotations of specific GRIN missense variants could be extrapolated to equivalent structural positions in other GluN subunits. First, the algorithm was experimentally validated, using an in silico library of GluN2B-equivalent GluN2A artificial variants and then was exhaustively applied to the full GRIN missense variants repertoire, consisting of 4525 variants. The algorithm revealed an absolute predictive power for GluN1, GluN2A and GluN2B subunits, both in terms of pathogenicity-association and functional impact. The structure-based computational algorithm duplicated the assignment of pathogenic GRIN variants, reduced by 30% the number of GRIN variants with uncertain pathogenesis and increased by 70% the number of annotated variants. Finally, the algorithm has been implemented into GRIN Database, providing a computational tool that accelerates GRIN missense identification.

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## P.53. AGE-ASSOCIATED CHANGES IN LIPIDOMIC PROFILE OF RAT FRONTAL CORTEX AND CEREBELLUM ARE REVERSED BY METHIONINE RESTRICTION APPLIED IN OLD AGE

**Sol J**[1], Cabré R[1], Mota-Martorell N[1], Martin-Garí M[1], Obis E[1], Galo-Licona JD[1], Portero-Otín M[1], Ayala V[1], Ferrer I[2], Jové M[1], Pamplona R[1]

[1] Department of Experimental Medicine, University of Lleida-Lleida Biomedical Research Institute (UdL-IRBLleida), 25198, Lleida, Spain.
[2] Department of Pathology and Experimental Therapeutics, University of Barcelona, L'Hospitalet de Llobregat, 08907, Barcelona, Spain.

Lipids are closely associated with brain structure and function. However, the potential changes in the lipidome induced by aging remain to be elucidated. In this study, we used chromatographic techniques and a mass spectrometry-based approach to evaluate age-associated changes in the lipidome of the frontal cortex and cerebellum from adult (8 months), aged (26 months), and aged submitted to a 8-weeks methionine restriction diet (MetR)-as an anti-aging intervention- male Wistar rats. The outcomes revealed that only small changes (about 10%) were observed in the lipidome profile in the cerebellum and frontal cortex during aging, and these changes differed, in some cases, between regions. Furthermore, a MetR diet partially reversed the effects of the aging process. Remarkably, the most affected lipid classes were ether-triacylglycerols, diacylglycerols, phosphatidylethanolamine N-methylated, plasmalogens, ceramides, and cholesterol esters. When the fatty acid profile was analyzed, we observed that the frontal cortex is highly preserved during aging and maintained under MetR, whereas in the cerebellum minor changes (increased monounsaturated and decreased polyunsaturated contents) were observed and not reversed by MetR. We conclude that the rat cerebellum and frontal cortex have efficient mechanisms to preserve the lipid profile of their cell membranes throughout their adult lifespan in order to maintain brain structure and function. Part of the small changes that take place during aging can be reversed with a MetR diet applied in old age.

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## P.54. ANTIOXIDANT MOLECULAR BRAIN CHANGES PARALLEL ADAPTIVE CARDIOVASCULAR RESPONSE TO FORCED RUNNING IN MICE

**Bartra C**[1], Jager LA[1], Alcarraz A[2], Meza A[2], Sangüesa G[2], Corpas R[1], Guasch E[2], Batlle M[2], Sanfeliu C[1]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona.
[2] Arrhythmia Unit, Hospital Clínic de Barcelona and IDIBAPS.

Physically active lifestyle has huge benefits for the health and well-being of people of all ages. However, excessive training can lead to severe cardiovascular events such as heart fibrosis and arrhythmia. In addition, strenuous exercise may impair brain plasticity. Here we investigate the presence of any deleterious effects induced by chronic exercise at the level of high intensity, although not reaching exhaustion. We analyzed cardiovascular, cognitive and cerebral molecular changes in young adult male mice submitted to treadmill running for eight weeks at moderate or high intensity regimens compared to sedentary mice. Exercised mice showed decreased weight gain that was significant for the high intensity level group. Exercised mice showed cardiac hypertrophy, but no morphological changes in the aorta. High-intensity training induced decrease in heart rate and increase in motor skills. However, it did not impair recognition or spatial memory and, accordingly, the expression of hippocampal neuroplasticity markers was maintained. Interestingly, catalase expression and proteasome enzymatic activity were increased in the cerebral cortex of the high-intensity trained group; both first-line mechanisms contributing to maintain redox homeostasis and prevent the accumulation of damaged proteins. Therefore, physical exercise at an intensity that induces adaptive cardiovascular changes in parallel increases antioxidant defenses to prevent brain damage and build resilience against neurodegeneration.

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## P.55. PHOSPHO-RNA-Seq IN PLASMA SUBFRACTIONS REVEALS SRNAS ENRICHED IN THE CENTRAL NERVOUS SYSTEM

**Solaguren-Beascoa M**[1], Gámez-Valero A[1,2], Escaramís G[1,2], Ortiz AM[3], Minguet C[3], Costa M[3], MartíE[1,2]

[1] Department of Biomedicine, Faculty of Medicine, Institute of Neurosciences, University of Barcelona, C/Casanova 143 (08036) Barcelona, Spain
[2] Biomedical Research Networking Center for Epidemiology and Public Health (CIBERESP), Ministry of Science and Innovation, Madrid, Spain
[3] Grifols Bioscience Research Group, Grifols, Barcelona, Spain

In recent years, RNA sequencing evolution has allowed to decipher and to analyze the complex composition of human transcriptome in a wide range of contexts. Specifically, the study of extracellular RNA in peripheral blood has opened new avenues in the search of disease and biological biomarkers.Diverse library and sequencing protocols have been described aiming to optimize the discovery of different RNA classes. Here, we intent to determine RNA abundance and diversity after applying the already described phospho-RNA pre-sequencing protocol (+PNK) (Giraldez et al., 2019) in comparison tothe standard small RNA sequencing approach (-PNK). To understand the added value of Phospho-RNA-seq, we applied this method to total plasma, and EV- and protein-enriched plasma subfractions. Plasma subfractions were isolated by size-exclusion chromatography (SEC), and sequencing data in PNK- and non-PNK treated RNAs were analyzed using SeqCluster and SeqBuster in-house bioinformatic tools. Most classes of sRNAs displayed an increased detection signal in response to PNK treatment, except miRNAs in all samples and tRNA-fragments in EVs and protein-enriched pools. Diferential expression (DE) analysis in total plasma, EV- and protein-enrichedfractions shows an increased detection of brain-enriched gene fragments in PNK treated RNAs, suggesting that PNK favors the detection brain dynamic changes linked to physiology and pathology. Overall, these results show that PNK treatment highlights an increased diversity in the sRNAs profiles in total plasma and SEC-derived EVs and protein-enriched pools. Furthermore, PNK treatment results in an increased detection of brain enriched gene fragments, offering an increased potential to detect transcriptomic perturbations associted with diseases of the central nervous system.

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## P.56. NOVEL OPTOGENETIC TOOLS TO MODULATE CAMP IN NEURONS: EFFECT'S ON HUNTINGTON'S DISEASE

**Sitjà-Roqueta L**[1,2,3], Coll-Manzano A[1,2,3], Conde-Berriozabal S[1,2,3], García-García E[1,2,3], Ramon-lainez A[1,2,3], Fernández-García S[1,2,3], Rodríguez MJ[1,2,3], Soriano J[4], Dalkara D[5], Möglich A[6], Alberch J[1,2,3], Masana M[1,2,3]

[1] Department of Biomedical Sciences, Institute of Neurosciences, School of Medicine and Health Sciences, Universitat de Barcelona. Barcelona, Spain.
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Barcelona, Spain.
[3] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED). Madrid, Spain
[4] Departament de Física de la Matèria Condensada, Universitat de Barcelona, Barcelona, Spain.
[5] Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France
[6] Universität Bayreuth, Bayreuth, Germany

In neurons, cAMP modulate metabotropic responses and induce many intracellular signalling pathways, including synaptic plasticity. Phytochrome photoreceptors can act as adenylate cyclase and produce cAMP in cells upon photoactivation, allowing spatio-temporal modulation of cAMP, which can be particularly relevant in neurodegenerative diseases such as Huntington's disease (HD). HD is characterized by motor disturbances, associated to a progressive disconnection of the cortico-striatal circuitry. Therefore, our main goal is to evaluate the potential of phytochromes as a novel tool to induce long-term neuronal plasticity in HD's specific brain circuits. We first explored cAMP levels at distinct brain regions and along HD progression by ELISA. Then, we evaluated how cAMP modulates neuronal activity dynamics by analysing Fluo4 calcium fluorescence intensity changes, as well as individual and collective spontaneous neuronal activity by Forskolin application, in WT and R6/1 mice primary cortical cultures at 14 DIV, using the NETCAL software. We observed that Forskolin increases the number of neurons firing collectively, while single neuronal activity (number of spikes, ISI, IBI) remains unaltered. Moreover, this effect was not observed in HD cultures. Accordingly, primary cultures from WT and HD were infected at 7 DIV with an AAV expressing Phytochromes (AAV9-CamKII-DdPAC-Flag-tag) and calcium dynamics induced by phytochrome activation are currently being analysed. We are also implementing fiber photometry tools to study phytochrome effects in vivo by using the calcium sensor GCamp6f and/or cAMP sensor Pink Flamindo. Altogether, these results contribute to the development of new approaches towards modulating brain activity and uncover circuit dynamics in Huntington's disease.

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## P.57. INSIGHT INTO AN EARLY-ONSET PARKINSON'S DISEASE MUTATION: IMPACT ON ADENOSINE A1-A2A RECEPTOR HETEROMERIZATION

**Sarasola LI**[1,2], Llinas del Torrent C[3], Fernández-Dueñas V[1,2], Ferré S[4], Pardo L[3], Ciruela F[1,2]

[1] Pharmacology Unit, Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, 08907 L'Hospitalet de Llobregat, Spain.
[2] Neuropharmacology & Pain Group, Neuroscience Program, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, 08907 L'Hospitalet de Llobregat, Spain.
[3] Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Universitat Autònoma Barcelona, Bellaterra, 08193 Barcelona, Spain.
[4] Integrative Neurobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA.

Adenosine is an endogenous autacoid purine nucleoside involved in several physiological functions. In the brain, it modulates neurotransmission through inhibitory adenosine A1 receptors (A1Rs) and stimulatory A2A receptors (A2ARs). These G protein-coupled ARs are involved in motor function and related to neurodegenerative diseases such as Parkinson's disease (PD). In line with this, a recent study associated a new autosomal recessive mutation (G279S) within the A1R gene to the development of early onset PD. Here, we aimed at investigating the impact of this mutation on receptors' structure and function. Our results revealed that the G279S A1R mutation does not alter receptor's ligand binding, constitutive activity or coupling to transducer proteins (i.e., Gαi and Gαq) in transfected cells. However, G279S mutation reduced A1R-A2AR heteromer formation and abolished the heteromer-dependent ligand-independent modulation that A1R exerts over the constitutive and agonist-induced activation of the A2AR. Interestingly, computational studies supported that the G279S A1R mutation could have a negative effect on the heterodimer interface stability. Overall, our results indicate that G279S mutation does not modify A1R canonical signalling, whereas it reduces the ability of A1R to act as a negative allosteric modulator of A2AR function.

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## P.58. UNRAVELLING THE DISTRIBUTION AND FUNCTION OF THE LIPID TRANSFER PROTEIN VPS13A IN THE BRAIN TO UNDERSTAND CHOREA ACANTHOCYTOSIS PATHOLOGY

**García-García E**[1,2,3], Chaparro-Cabanillas N[1,2,3], Coll-Manzano A[1,2,3], Carreras-Caballé M[1,2,3], Giralt A[1,2,3], del Toro D[1,2,3], Masana M[1,2,3], Alberch J[1,2,3], Rodríguez MJ[1,2,3]

[1] Department of Biomedical Sciences, Institute of Neurosciences, School of Medicine and Health Sciences, Universitat de Barcelona. Barcelona, Spain.
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Barcelona, Spain.
[3] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED). Madrid, Spain

Chorea-acanthocytosis (ChAc) is caused by a VPS13A gene mutation leading to marked reduction or absence of VPS13A protein. ChAc patients show progressive movement disorders such as chorea and dystonia. The main neuropathologic feature in VPS13A mutations is a selective degeneration of the striatum, however, little is known about the VPS13A expression in the brain. There is also a poor knowledge about VPS13A function in neural cells. Thus, the objectives of this work are a) to assess the time course and the regional expression of vps13a gene in the mouse brain and b) to study the vps13a interaction partners. Single cell RNA showed that vps13a is present in mature neurons and qPCR revealed that vps13a expression is stable over time. Then, we used fluorescence in-situ hybridization and immunohistochemistry to determine the distribution of vps13a mRNA and protein in mouse brain from embryonic stages to adulthood. In the adult mouse brain, we found a widespread distribution of vps13a, with different staining intensity profiles between nuclei. In general, the mRNA localization resembled that of the protein one with an enrichment in the pons, cerebellum and hippocampus. We found moderate staining in the cortex and in the most thalamic and hypothalamic nuclei. Interestingly, we found weak staining in the basal ganglia nuclei. We observed vps13a staining in glutamatergic, GABAergic and cholinergic neurons. Not only neurons but also some glial cells expressed chorein. The levels of vps13a protein were not modulated neither by pilocarpine, amphetamine nor ketamine treatments, suggesting that VPS13A has structural and stable role in neural cells. We also evaluated the vps13a interactome through a specific protein immunoprecipitation from mouse cerebral cortex followed by mass spectrometry. Vps13a interacts with lipid metabolism proteins. Understanding the brain tissue distribution, expression and protein interacting partners can provide novel insights toward the knowledge of ChAc pathophysiology.

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## P.59. CBD AS A POTENCIAL NEUROPROTECTIVE AGENT

**Raïch I**[1,2], Lillo A[1,2], Lillo J[2,3], Pérez-Olives C[3], Rivas-Santisteban R[2,3], Rebassa JB[1], Navarro G[1,2]

[1] Department of Biochemistry and Physiology. School of Pharmacy. University of Barcelona, 08028 Barcelona. Spain.
[2] Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, C/Sinesio Delgado, 4, 28029 Madrid, Spain.
[3] Molecular Neurobiology Laboratory. Department of Biochemistry and Molecular Biomedicine. University of Barcelona, Diagonal 643, 08028 Barcelona, 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.

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## P.60. MODULATION OF GUT MICROBIOTA IS ASSOCIATED WITH THE NEUROPROTECTIVE EFFECTS OF SPRAY-DRIED PORCINE PLASMA

**Rosell-Cardona C**[1], Miró L[1], Amat C[1], Polo J[2], Moretó M[1], Pérez-Bosque A[1]

[1] Departament de Bioquímica i Fisiologia (Secció de Fisiologia), Facultat de Farmàcia i Ciències del'Alimentació, i Institut de Nutrició i Seguretat Alimentària, Universitat de Barcelona (UB), 08028Barcelona
[2] APC-Europe S.L.U., 08403 Granollers

The progression of Alzheimer's disease is associated with alterations in the gut microbiota. Dietary supplementation with spray-dried porcine plasma (SDP) reduces neuropathological AD hallmarks in SAMP8 mice. The aim of this work was to know whether modulation of the gut microbiota can exert a role in the neuroprotective effects of SDP supplementation. The experiments were performed in 2- month-old SAMP8 mice fed a standard diet and in 6-month-old SAMP8 mice fed a control diet or an 8% SDP supplemented diet for last 4 months. Cognitive performance was evaluated using the Novel Object Recognition test; BDNF abundance in the brain cortex was determined by Western Blot; and cytokines were quantified by Real-Time PCR and a commercial kit. Faecal microbiota analysis was performed using Illumina MiSeq platform. Senescence reduced short- and long-term memory as well as cortical BDNF abundance, while SDP supplementation prevented these effects (all, $p<0.05$). Aging augmented the expression of the pro-inflammatory cytokine Il-1β and diminished the concentration of the anti- inflammatory IL-10 in the cortex tissue (both, $p<0.05$). Senescence also increased serum concentration of IL-1β and TNF-α and colonic expression of both cytokines (all, $p<0.05$), which were prevented by SDP supplementation prevented this aging-associated inflammation on the brain, systemic and colon (all, $p<0.05$). Furthermore, senescence reduced the abundance of probiotic bacteria such as Lactobaccillus, and Pediococcus, and increased Erysipelhotrix genera, which is associated with inflammation (all, $q <0.05$). These effects were prevented by SDP supplementation (all, $q <0.05$). In conclusion, the neuroprotective effects of SDP could be exerted by promoting the abundance of health-beneficialbacteria and enhancing mucosal and systemic anti-inflammatory pathways.

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## P.61. PERSONALIZED TREATMENTS SERVICE IN HOSPITAL SANT JOAN DE DÉU NEUROLOGY DEPARTMENT: A NEW ERA IN THE TREATMENT OF NEUROPEDIATRIC DISORDERS

**Oyarzabal A**, García Cazorla A

Synaptic Metabolism Lab. Department of Neurology. Institut de Recerca Sant Joan de Déu, CIBERER and metabERN. Barcelona, Spain

Neuropediatric diseases are complex conditions with an intricate molecular pathophysiology. The personalized study of the underlying cellular alterations can be the best (if not the only) way to treat these disorders. In the Neurology Department of Hospital Sant Joan de Déu we have added a “personalized medicine service” to our portfolio, for the study and treatment of complex neuropediatric diseases with genetic origin. The service brings together neuropediatricians and molecular neuroscientists that analyse each case in an individualized fashion to propose a personalized therapeutic option. The patient is first visited by the neuropediatrician, and the case is assessed by the whole team, that shape an *ad hoc* defined project to test different therapeutic options. Those experiments will be conducted in the upcoming monthsand will conclude with a set of potential therapeutic alternatives, that will be transferred to the patient basedon the neuropediatrician evaluation.

In the last years (since the Service has started) we have studied over 50 different patients. To illustrate thisapproach to the diagnosis and treatment of neuropediatric diseases we present a selection of over 15 patientsrecently studied. All the patients were referred to our service due to a complex neurological presentation -including paediatric parkinsonism and movement disorders, and/or suggestive of neurotransmitter defects.The selection of patients includes defects on neurotransmission, mitochondrial and autophagy-related disorders, metabolic defects and transport disorders. Two main scenarios were faced:

a)Molecular confirmation of a genetic diagnosis for discordant presentations: These cases concludewith both the confirmation of the patient's diagnosis and description of either a new disease or a new presentation of a known disease.
b)Proposal of new therapeutic targets: n-of-1 trials. When an actionable target was identified, we defined a personalized project to test different interventions (either drugs or nutraceuticals) on patient cells, identifying the best option. The lab results were transferred to the clinic, designing an-of-1clinical trial, altering the natural history of the disease

Personalized and multidisciplinary study of the disease results in the identification of new therapeutic alternatives for neuropediatric disorders.

## MENTAL AND BEHAVIORAL DISORDERS

### P.62. NEUROIMMUNOLOGICAL IMPLICATIONS OF THE IKAROS FAMILY IN SCHIZOPHRENIA

**Ballasch I**[1,2,3], López-Molina L[1,2,3], Alberch J [1,2,3,4], Arranz B[5], Canals JM[1,2,3,4], Giralt A[1,2,3,4]

[1] Departament de Biomedicina, Facultat de Medicina, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain.
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.
[3] Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain.
[4] Production and Validation Center of Advanced Therapies (Creatio), Faculty of Medicine and Health Science, University of Barcelona, Barcelona, Spain.
[5] Parc Sanitari Sant Joan de Déu, CIBERSAM, Barcelona, Spain.

Schizophrenia is a severe neuropsychiatric disease affecting approximately 1% of the world population. It is characterized by a variety of symptoms that are classified into three broad categories: positive, negative and cognitive. Lately, alterations in the immune system have been linked to the development of schizophrenia, rising the idea of an aberrant cross-talk between the nervous system and the immune system during this disease.

Some members of the Ikaros family of transcription factors are known to be required for the proper development and function of the immune system. We found three of these members (Ikaros, Helios and Aiolos) to be altered in PBMCs of a cohort of schizophrenic patients. Concretely, Ikaros and Helios were downregulated. Interestingly, in a mimicking experiment in mice, we have also found schizophrenia-like symptoms expression in a doble mutant mouse model (Ikaros +/-; Helios +/-) corresponding to the three symptom categories of schizophrenia. Specifically, we found hyper-sensitivity to psychostimulants, impaired social skills and decreased long-term recognition memory. Taking in account these findings our objective is to continue exploring the implication of this family in the development and course of schizophrenia using translational in vivo and in vitro approaches.

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## P.63. LINKING ENDOPLASMIC RETICULUM STRESS TO DEPRESSION: INVOLVEMENT OF EIF2α PATHWAY

**Miquel-Rio L**[1,2,3], Sarriés-Serrano U[1,2,3], Paz V[1,2,3], Campa L[1,2,3], Bortolozzi A[1,2,3]

[1] Institut d'Investigacions Biomèdiques de Barcelona (IIBB), Spanish National Research Council (CSIC), Barcelona, Spain
[2] Institut d'Investigacions August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[3] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), ISCIII, Madrid, Spain

Aims: Depression is a devastating mood disorder that causes profound disability worldwide. Despite the growing number of antidepressant medications available, treatment options for depression are limited. Therefore, it is imperative to understand the etiology and pathophysiology of depression to discover novel therapeutic targets of action. Here, we explore how endoplasmic reticulum (ER) stress might play an important role in the pathophysiology of depression and how the antidepressant ketamine actions involve ER pathways
Methods: We generated a mouse model of ER stress in serotonin (5-HT) neurons using the stressor tunicamycin (200 µg/µl). We examined ER/UPR pathway markers by Western blot, neuroplasticity gene expression (BDNF, TrkB, VEGF, Neuritin, PSD95, and Zif268) by in situ hybridization, 5-HT release by microdialysis, and behavioral depressive-like phenotype. Ketamine (10 mg/kg, i.p.) was used to reverse the ER stress-induced depressive mouse model.
Results: Tunicamycin-induced ER stress in 5-HT neurons left a time-dependent increase in GRP78 and CHOP protein levels. In addition, increased phosphorylation of eIF2α and eEF2 was found, suggesting activation of PERK pathway. Tunicamycin-treated mice exhibited an anxious/depressive phenotype, reduced 5-HT release in the medial prefrontal cortex, and changes in neuroplasticity gene expression in 5-HT projection areas. A single dose of ketamine reversed the depressive phenotype 30 minutes later, which is associated with reduced levels of phosphorylated eIF2α and recovery of BDNF expression.
Conclusions: The results strongly indicate that ER stress and UPR may represent cellular pathogenic mechanisms in the development of mood disorders and that eIF2α pathway is central for the antidepressant activity of ketamine.

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## P.64. EXPLORING THE INTERACTION OF CANNABIS USE AND ENDOCANNABINOID RECEPTOR GENES ON THE PERCEPTUAL ORGANIZATION IN FIRST-EPISODE PSYCHOSES

Oscoz-Irurozqui M[1,2], Guardiola-Ripoll M[1,3], **Almodóvar-Payá C**[1,3], Guerrero-Pedraza A[4], Hostalet N[1], Pomarol-Clotet E[1,3], Fatjó-Vilas M [1,3,5]

[1] FIDMAG Germanes Hospitalàries Research Foundation. Barcelona, Spain.
[2] CSM IIA - Milagrosa y II Ensanche. Pamplona, Spain
[3] CIBERSAM (Biomedical Research Network in Mental Health); Instituto de Salud Carlos III, Madrid, Spain.
[4] Hospital Benito Menni CASM; Sant Boi de Llobregat, Spain.
[5] Department of Evolutionary Biology, Ecology and Environmental Sciences; Faculty of Biology; University of Barcelona, Barcelona, Spain.

Background. Cannabis use has been consistently associated with the risk for psychosis. Also, its effects on cognition have been largely explored, with the domains of verbal memory, attention and executive functioning being affected most consistently (Broyd et al., 2016; Cohen & Weinstein, 2018). Some reports in psychotic samples show better cognitive status in cannabis users (CU) than non-user patients (CNU). Here we aimed to evaluate the association of cannabis use and its interplay with Cannabinoid Receptor 1and 2 genes (CNR1 and CNR2) on cognition in patients with a first episode of psychosis (FEP).
Materials and Methods. The sample comprised 50 Caucasian individuals with a first episode of psychosis (mean age(sd)=26.14(6.55) years, 76% males, 58% cannabis users). There were no differences in age, sex, premorbid IQ and antipsychotic dose between cannabis users and non-users. Psychotic symptoms were evaluated with the PANSS scale, and scores of positive, negative, disorganized and depressive syndromes were obtained. Also, the general functioning was evaluated with the GAF). Genetic variability was assessed by genotyping one Single Nucleotide Polymorphism (SNP) in the CNR1 gene (rs1049353) (qPCR, TaqMan).
Results. Genotypic frequencies were in Hardy-Weinberg equilibrium, and they did not differ between cannabis users and non-users. CU displayed better than CNU in manipulative abilities, measured by the matrix test of WAIS (CU (9.45(0.71) and CNU 7.00(0.89), p=0.041). The interaction model did not evidence a combined effect of CNR1 nor CNR2 and cannabis use on the clinical outcome.
Conclusions. Our finding of a better cognition (measured by the matrix test of WAIS) in CU psychosis patients, when compared with CNU, converges with other previous data. It has been suggested that those patients could be neurocognitively less damaged and have a lower intrinsic vulnerability, but developed psychosis after an early start of cannabis consumption (Løberg et al., 2014; Yücel et al., 2012).

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## P65. IMPAIRED DYNAMICS IN SYNAPTIC SIGNALLING TRIGGERED BY SLEEP/WAKE CYCLE IN SYNGAP+/- MICE

**del Castillo-Berger D**[1,2], Peñuela A[1] , Gou G[1,2], Bayés A[1,2]

[1] Molecular Physiology of the Synapse Laboratory, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain.
[2] Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain

SYNGAP1 is a gene that codes for the 'Synaptic Ras/Rap GTPase activating protein' (SynGAP). In adults, SynGAP is very restricted to the postsynaptic density (PSD) of forebrain excitatory synapses, where it is one of the most abundant proteins. SynGAP inhibits Ras and Rap GTPases, which modulates two main processes involved in synaptic plasticity: AMPA receptor trafficking and dendritic spine size.

De-novo mutations in SYNGAP1 represent one of the most prevalent monogenic forms of Intellectual Disability (ID) (Orphanet code: ORPHA178469; OMIM code: #612621), with patients typically displaying moderate or severe ID and a generalized form of epilepsy. Autism spectrum disorders, attention deficits, impulsivity, mood and sleep disorders, mild hypotonia and global developmental delay are also present. The Synaptic Homeostasis Hypothesis (SHY) proposes that the fundamental function of sleep is the restoration of synaptic homeostasis, which is challenged by synaptic strengthening triggered by learning during wake. Since SYNGAP1 mutations are associated with sleep disorders, increased epileptic activity during sleep and excessive basal potentiation of synapses, our hypothesis is that homeostatic downscaling of synapses during sleep is impaired in individuals carrying SYNGAP1 mutations.

To test our hypothesis, we isolated PSDs from cortices of WT and Syngap1+/- mice collected both during sleep and wake periods and studied the levels of several molecular markers associated with synaptic potentiation. As expected, we found an increase in synaptic strength markers in WT mice during wake compared to sleep, but not Syngap1+/- mice. Comparisons between WT and Syngap1+/- during sleep revealed increased basal levels of synaptic potentiation markers in the PSDs of Syngap1+/- mice, while there were little or no differences during wake between genotypes. Taken together, this suggests that, while WT mice undergoes synaptic strengthening during wake and downscaling during sleep, Syngap1+/- synapses are locked in a potentiated state and they are incapable of undergoing physiologic downscaling during sleep.

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## P.66. UNDERSTANDING PSYCHOTIC DISORDERS THROUGH THE PRISM OF FACE AND BRAIN INTERRELATED DEVELOPMENT

**Hostalet N**[1], Gonzàlez R[2], Canales-Rodríguez EJ[1,2,4], Salgado-Pineda P[1,3], Salvador R[1,3], Pomarol-Clotet E[1,3], González A[5], Sevillano X[5], Martínez-Abadías N[2] and Fatjó-Vilas M[1,2,3]

[1] FIDMAG, Sisters Hospitallers Research Foundation, Barcelona
[2] University of Barcelona, Department of Evolutionary Biology, Ecology and Environmental Sciences, Barcelona, Spain
[3] CIBERSAM, Biomedical Research Network in Mental Health; Instituto de Salud Carlos III, Madrid, Spain
[4] Signal Processing Lab (LTS5), École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.
[5] GTM–Grup de Recerca en Tecnologies Mèdia, La Salle, Universitat Ramon Llull, Barcelona, Spain

Background: Brain and facial tissues are both derived from the same ectodermal layer and they are developed in an interrelated way under the influence of the Sonic Hedgehog (Shh) signalling pathway. Then, facial traits and brain morphology together with genetic markers involved in craniofacial and brain development, emerge as good candidates to predict vulnerability for disorders in which neurodevelopmental processes may be compromised, such as schizophrenia (SZ) and bipolar disorder (BD). We have analysed the association of SHH, SUFU and GLI3, key elements of Shh signalling pathway, with SZ and BD and how they modulate face and brain morphological relationships.

Methods:The sample comprised 113 SZ patients, 129 BD patients and 195 healthy controls (HC). We genotyped 3 SNPs (SUFU-rs10786679, SHH-rs10949808, GLI3-rs3735361) and conducted a case-control genetic association approach. From 53 SZ patients, 22 BD patients and 42 HC we obtained facial 3D reconstructions and neuroanatomical cortical measures from MRI scans. The brain-face-genes correlations were analysed using Geometric Morphometrics and multivariate statistics analyses.

Results: The genetic association analyses revealed allelic and recessive effects of the T allele of the SHH- rs10949808 on the risk for SZ (p=0.031 and p=0.028, respectively). The A allele of the SUFU-rs10786679 was associated with BD (under allelic (p=0.004) and dominant (p=0.005) models). Regarding morphometric analyses, we detected a significant association of superiorfrontal region volume with facial shape (p=0.05) when comparing SZ and HC subjects. A combined effect of GLI3 genotype, superiorfrontal region area and diagnosis of BD on facial shape was found (p=0.03), which explained 3.2% of facial shape variance.

Discussion: Overall, the association of genes implicated in face and brain development, such as SHH and SUFU with SZ and BD, and the influence of brain measures on facial shape modulated by GLI3, support the notion of facial shape as an indirect neuroanatomical marker for SZ and BD.

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## SENSORY AND MOTOR SYSTEMS

### P.67. THE INCREASED ANTIALLODYNIC EFFECTS OF MORPHINE IN ANIMALS WITH NEUROPATHIC PAIN TREATED WITH HYDROGEN SULFIDE DONORS

**Bai X**, Batallé G, Pol O

Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau, Hospital de la Santa Creu i Sant Pau and Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

Recent studies reveal that the administration of slow-releasing hydrogen sulfide ($H_2S$) donors increases the antinociceptive effects of δ-opioid receptor (DOR) agonists in inflammatory pain, thus showing the potential benefit of the co-administration of $H_2S$ donors with DOR agonists. Nevertheless, the possible improvement on the analgesic effects of the μ-opioid receptor (MOR) agonist, morphine, induced by its co-treatment with DADS (diallyl disulfide) or GYY4137 (morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate dichloromethane complex), two slow-releasing $H_2S$ donors during neuropathic pain, is still unknow. Therefore, in a neuropathic pain model provoked by the chronic constriction of the sciatic nerve (CCI) in male C57BL/6J mice, we evaluated the effects of DADS and GYY4137 on 1) the antiallodynic actions induced by the systemic and local administration of morphine; 2) the oxidative stress and apoptotic responses induced by CCI in septum medial (SM) and dorsal root ganglia (DRG) and 3) the expression of MOR in DRG. Our results revealed that both DADS and GYY4137 increased the antinociceptive effects of morphine by enhancing the expression of MOR in the DRG. In addition, both treatments inhibited the oxidative stress and apoptotic responses provoked by nerve injury in SM and/or DRG. Moreover, the analgesic effects of DADS and GYY4137 were reversed with the administration of naloxone. This study reveals that $H_2S$ potentiates the antinociceptive properties of morphine and proposes their co-administration as interesting option for the management of neuropathic pain.

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## P.68. VESTIBULAR TOXICITY OF 3,3-IMINODIPROPIONITRILE (IDPN) IN FEMALE RATS

**Palou A**[1,2], Llorens J[1,2]

[1] Institut de Neurociències, Departament de Ciències Fisiològiques, Universitat de Barcelona, Hospitaletde Llobregat
[2] Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospitalet de Llobregat

The vestibular system is the sensory system that encodes head accelerations. These accelerations are detected by transducing cells, known as hair cells (HCs). There are two types of HCs, type I (HCI) and typeII (HCII). In each inner ear, vestibular HCs are found in five sensory epithelia: one crista in each semicircular canal, and two maculae in the utricle and saccule. As a useful research tool, graded lesions of the vestibular sensory epithelia can be generated using ototoxic compounds. In male rats, exposure to 3,3'-iminodipropionitrile (IDPN) is a good model to causevestibular loss by both acute and chronic exposure. Previous studies have discovered differences between sexes in IDPN ototoxicity, but only male rats have been used for ototoxicity research with this compound. The objective of this study was to establish IDPN ototoxicity models in female rats.Female Long-Evans rats were exposed to sub-acute doses (0, 150, 200 or 300 mg/Kg per day x 3 consecutive days) of IDPN. At several time points after ototoxic exposure, we tested for vestibular function using high speed video to record two anti-gravity reflexes (tail lift and air righting reflexes). Three weeks after administration, the vestibular sensory epithelia were collected and processed to estimate the loss of HC in each epithelium. Cell counts were obtained in confocal microscopy images.
In a dose-dependent manner, IDPN caused a decrease in tail-lift angles, an increase in air-righting times, and a decrease in the number of HCs remaining in the epithelia. Cell loss was greater in the crista, followed by the utricle and the saccule. The loss was more prominent in central zones of the epithelia. Inaddition, a higher sensitivity to IDPN toxicity was observed in HCI cells compared to HCII. In comparison to previous male data, females required higher doses to attain a similar lesion.

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## P.69. CELLULAR COMPOSITION AND MATURATION OF THE VESTIBULAR SENSORY EPITHELIA IN THE RAT

**Borrajo M**[1], Sedano D[2], Llorens J[1]

[1] Institut de Neurociències and IDIBELL, Departament de Ciències Fisiològiques, Universitat de Barcelona, Hospitalet de Llobregat
[2] Departament de Ciències Fisiològiques, Universitat de Barcelona, Hospitalet de Llobregat

Vestibular hair cells (HC) of the inner ear are the mechanoreceptors responsible for proprioception and equilibrium. They have been classically divided into two distinct types by morphological criteria: type I (HCI) and type II (HCII). HCI are goblet-shaped and enveloped by a calyx-like nerve termination, while HCII are cylindrical and contacted by bouton nerve terminations. Prior studies in the rat estimated that HCs comprise 55% HCI and 45% HCII. However, this classification was not based on specific molecular markers. To provide an updated description of the composition and development of the vestibular organs, we collected utricles, saccules, and cristae of Long-Evans rats on postnatal days (P) 0, 1, 4, 7, 10, 14, 28 and 60 and analyzed the differentiation of HC into type I or II by using specific molecular markers for each cell type (SPP1 for HCI, calretinin for HCII, and Myo7a for all HC) and manually obtaining whole organ cell counts. Additionally, we validated SPP1 and the stereocilia protein ATP2B2 as HCI markers by examining their association with the previously established calyx marker, CASPR1. Our data show that, in adults, cells expressing SPP1 also express ATP2B2 and associate with CASPR1+ calyces. The total number of HC in vestibular organs increases during the first postnatal days, up to P4. The adult HCII marker calretinin is expressed during the first postnatal days in 98% of HC, both co-expressed and not with SPP1. Calretinin expression declines after day 4 to remain in approximately 30% adult HC. SPP1 is expressed in roughly 45% HC at P0, and this percentage increases to 65% in the adult rat. At all ages, a small percentage (3-6%) of HCs that express neither SPP1 nor calretinin are HCII that do not associate with a CASPR1+ calyx. All variables were similar in cristae, utricles, and saccules.

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## P.70. DISMANTLEMENT OF THE CALYCEAL JUNCTION IS A GENERAL RESPONSE TO STRESS OF THE VESTIBULAR SENSORY EPITHELIUM

**Llorens J**[1], Borrajo M[1], Callejo A[2], Pérez-Grau M[2], Amilibia E[2], Roca-Ribas F[2], Castellanos E[3], Maroto AF[1]

[1] Institut de Neurociències and IDIBELL, Departament de Ciències Fisiològiques, Universitat de Barcelona, Hospitalet de Llobregat

[2] Servei d'Otorinolaringologia, Hospital Germans Trias i Pujol, Badalona

[3] Clinical Genomics Research Unit. Institut de Recerca Germans Trias i Pujol (IGTP), Badalona

In previous studies, we discovered that sub-chronic exposure of rodents to an experimental ototoxic compound, IDPN, causes reversible effects in the contact between type I hair cells (HCI) and calyx afferent terminals of the vestibular sensory epithelium. These include early dismantlement of the calyceal junction and synaptic uncoupling. The aim of this study was to evaluate the relevance of this pathology to clinically-relevant stress conditions. We first studied in rats the effects of sub-chronic exposure to streptomycin, a well-known human ototoxin. Starting at day 21, rats received streptomycin once or twice a day for 3 to 8 weeks. Vestibular function was assessed at regular time intervals using the tail-lift reflex. After exposure, the vestibular sensory epithelia were studied by SEM, TEM, and confocal microscopy. We recorded a reversible loss of vestibular function. Although this was associated with a partial loss of HC, the calyces encasing the surviving HCI showed decreased expression of CASPR1, denoting calyceal junction dismantlement. Streptomycin rats also showed reduced numbers of post-synaptic (PSD95) and pre-synaptic (ribeye) puncta per cell, as well as KCNQ4 mislocalization. In animals allowed to recover after the end of the treatment, recovery in CASPR1 label revealed the rebuilding of the junction. We also studied human sensory epithelia from vestibular schwannoma patients who had the tumor removed by an ablative trans-labyrinthine approach. These tumors have been shown to cause inflammatory stress to the sensory epithelia. Using CASPR1 labeling, we observed that some human epithelia had intact calyceal junctions, but other patients presented patched label denoting dismantlement of the calyceal junction. We conclude that dismantlement of the calyceal junction is a common response triggered by chronic stress in the vestibular sensory epithelium. This effect precedes HC loss, is reversible, and may explain, at least in part, reversible function loss.

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## P.71. AGEING-ASSOCIATED ALTERATIONS IN BDNF/TRKB PATHWAY INVOLVING PKC AND SYNAPTIC TARGETS OF THE NEUROMUSCULAR JUNCTION

**Balanyà-Segura M**, Hurtado E, Just-Borràs L, Polishchuk A, Cilleros-Mañé V, Tomàs M, Tomàs J, Lanuza MA, Garcia N

Unitat d'Histologia i Neurobiologia, Depertament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus.

Ageing of the population in many countries around the world is one of the important challenges of modern society. Age-related conditions, such as sarcopenia or the impaired neuromuscular transmission are, currently, among the leading causes of morbidity and death worldwide. The bidirectional communication between the nervous system and muscles is fundamental for their health. Despite that the molecular mechanisms behind its regulation remain largely unknown, several signaling pathways that control neurotransmission, are crucial for this interaction. The muscle-derived Brain-derived-neurotrophic-factor (BDNF) acting through its receptor, Tropomyosin-related-kinase-B (TrkB) is well known for its neuroprotective functions and has been demonstrated that enhances presynaptic downstream effectors PKC isoforms and exocytotic proteins of synaptic vesicles (SNAP25 and Munc18-1) at the neuromuscular junction (NMJ). However, whether this signaling pathway is compromised in the aged neuromuscular system has not been analysed yet.

To address it, we analyze by Western Blot how BDNF/TrkB pathway could be altered in aged Extensor digitorum longus (EDL) rat muscles. Results obtained show that in EDL muscles this signaling cascade is strongly perturbed in aging. Phosphorylation status of PKC isoforms and their exocytotic targets of synaptic vesicles that directly regulate neuromuscular activity are decreased in aged muscles. These results are in concordance with the age-related impairment of the neurotransmission.

Thus, considering these results, therapeutic strategies recovering this signaling pathway function should improve NMJ functionality, slowing down the aging of the neuromuscular system and, thus, improving quality of life of aged people.

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## P.72. TRESK BACKGROUND POTASSIUM CHANNEL MODULATES THERMAL SENSITIVITY IN MICE

**Pujol-Coma A**[1], Eriksson E[1], Callejo G[1,2], Comes N[1,2], Gasull X[1,2]

[1] Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona,Spain

TRESK is a background potassium channel activated by intracellular calcium through the phosphatase Calcineurin. The channel is highly expressed in distinct populations of primary sensory neurons involved in nociception, where it modulates their excitability. We previously described that TRESK ablation induces an increase in mechanical and coldsensitivity, and its downregulation after nerve injury has been linked to chronic pain. Interestingly, Calcineurin inhibition by the drug Tacrolimus (FK-506) can induce cold allodynia and hyperalgesia in human patients as a side effect. Here, we explore the behavioral effects of Tacrolimus and the role of TRESK in the modulation of heat and cold sensitivity. First, we proved that the expression of major background potassium andTRP channels expressed in primary sensory neurons is not modified by TRESK ablationin the TRESK knockout mice model used in our studies. We also found that, in addition to its high expression in sensory ganglia, TRESK is also expressed at lower levels in spinal cord, brain, cerebellum and hippocampus. Behavioral studies show that, when exposed to a cold ramp, TRESK knockout mice present nocifensive behaviors in responseto higher temperatures than wild-type animals. Interestingly, only wild-type male mice treated with Tacrolimus present an enhanced sensitivity to cold. To better understand thecellular basis of this differences, responses to cold stimuli of different populations of primary sensory neurons are under study. TRESK ablation also induces an increase in heat sensitivity in male mice, but not in females. Besides, heat sensitivity of female miceis increased after Tacrolimus treatment in a TRESK-independent manner. In summary, TRESK modulates cold sensitivity and its indirect inhibition by Tacrolimus in wild-typemice resembles the cold allodynia observed in knockout animals. Given the channel's role in thermal sensitivity modulation and its mainly peripheral expression, TRESK activation is a potential therapeutic approach for the treatment of Tacrolimus-induced allodynia and hypersensitivity to painful stimuli.

## P.73. TRESK BACKGROUND K+ CHANNEL REGULATES SENSORY NEURON EXCITABILITY AND CONTRIBUTES TO MECHANICAL AND COLD PAIN

Castellanos A[1,2], Andres-Bilbe A[1,2], Negm A[3,4], Callejo G[1], Noël J[3,4], Gasull X[1,2], **Comes N**[1,2]

[1] Institute of Neurosciences, Universitat de Barcelona, Barcelona, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[3] Université Côte d'Azur, CNRS UMR 7275, Institut de Pharmacologie Moléculaire et Cellulaire, France
[4] LabEx Ion Channel Science and Therapeutics, Valbonne, France.

TRESK (K2P18.1) is a background K+ channel highly expressed in spinal cord, dorsal root and trigeminal ganglia sensory neurons, where it is involved in modulating sensory neuron excitability and firing. Changes in TRESK expression and function have been reported to enhance nociceptor excitability after injury or inflammation. To determine its role in sensory transduction, we compared the excitability and membrane properties of small/medium-sized sensory neurons in whole cell patch clamp recordings of cultured DRG neurons from wild type and TRESK knockout mice, which presented a reduced action potential threshold, increased membrane resistance and enhanced repetitive firing upon depolarization. Recordings of skin nociceptive fibers showed strong activation in response to cold in the absence of TRESK. In agreement, behavioral experiments in TRESK ko mice revealed a decreased mechanical threshold to von Frey hairs and an enhanced cold sensitivity. No significant changes were found for thermal sensitivity to warm or hot temperatures. Nocifensive behavior after capsaicin injection was unaltered while the response to AITC was slightly diminished. Interestingly, TRESK ko mice presented a reduced response to hypertonic and hypotonic stimuli even after sensitization with PGE2. During inflammation, ko mice showed a decreased phase I response in the formalin test, while phase II was unaltered. In the CFA-induced inflammatory model, both mechanical and thermal sensitivity were enhanced compared to wt animals. Mechanical and thermal hyperalgesia were also enhanced in the sciatic nerve cuffing model of neuropathic pain. Finally, the oxaliplatin-induced cold sensitization was absent in ko mice, probably due to the already enhanced cold sensitivity. In summary, TRESK has a significant contribution regulating the excitability of certain populations of sensory neurons mainly involved in mechanical and cold pain sensing. Moreover, down-regulation of its expression as occurs after nerve injury might contribute to the generation of the hyperalgesia and allodynia observed during chronic pain.

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Carles A. Saura, Ph.D
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