X SIMPOSI DE NEUROBIOLOGIA

6 i 7 d'octubre de 2016

Institut d'Estudis Catalans, Barcelona

Programa i resums de les comunicacions

Amb el patrocini de:

Societat Catalana de BIOLOGIA

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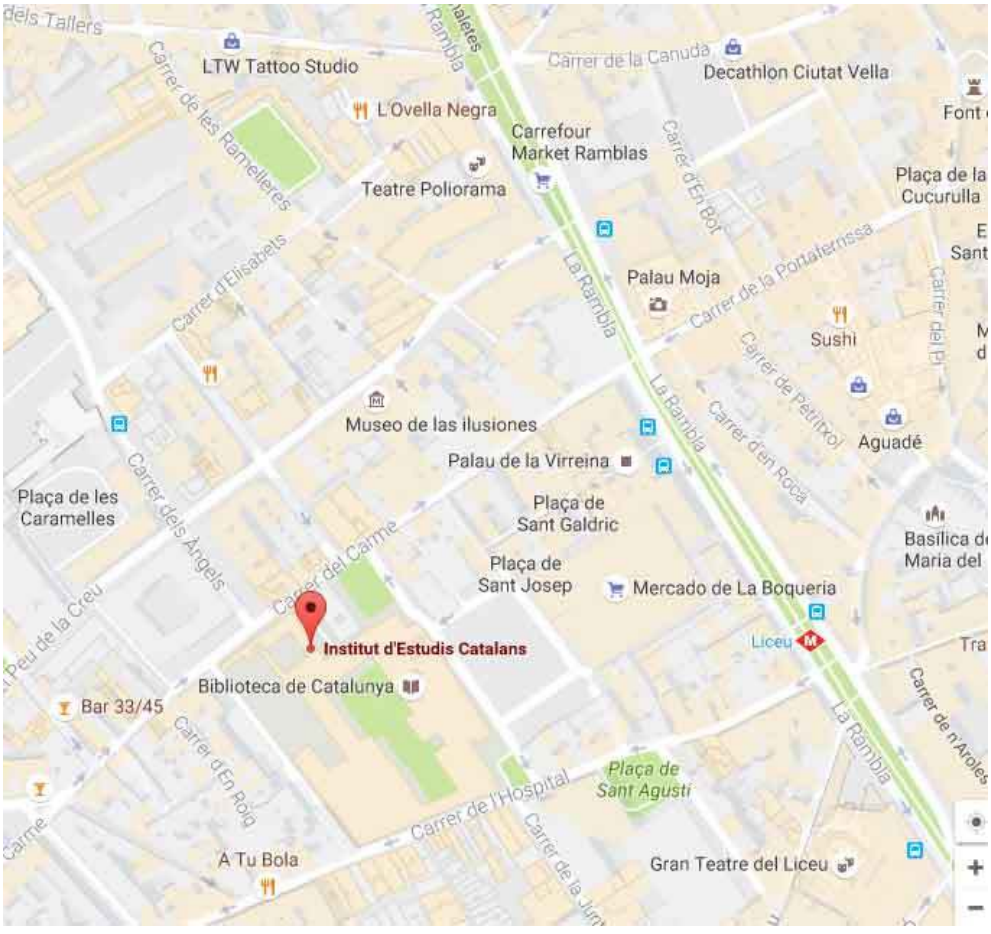

INSTITUT D'ESTUDIS CATALANS
Carrer del Carme, 47
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Societat Catalana de BIOLOGIA

PROGRAMA

Dia 1: 6 d'octubre de 2016		Dia 2: 7 d'octubre de 2016	
8.30- 8.45 h	**Registre i recollida de material**		
8.45 h	**Benvinguda i presentació del simposi** Sala: Prat de la Riba		
9.00- 10.45 h	**Sessió 1A** Comunicacions Orals **Cèl.lules glials i neuroinflammació** Sala: Prat de la Riba **Sessió 1B** Comunicacions Orals **Desenvolupament, neurogènesi i cèl·lules mare** Sala: Pere i Joan Coromines	9.00- 10.45 h	**Sessió 3A** Comunicacions Orals **Malalties neurodegeneratives II** Sala: Prat de la Riba **Sessió 3B** Comunicacions Orals **Sistemes motor i sensorial i noves teràpies** Sala: Pere i Joan Coromines
10.45- 12.00 h	Cafè i sessió de **pòsters P1-42**	10.45- 12.00 h	Cafè i sessió de **pòsters P43-85**
12.00- 13.00 h	**Conferència plenària 1**: **Rüdiger Klein** **Max-Planck Institute of Neurobiology (Germany)** Títol: «Axon guidance and cues during neuronal development» Sala: Prat de la Riba	12.00- 13.00 h	**Conferència plenària 3**: **Javier De Felipe** **Univ. Politècnica de Madrid, Instituto Cajal** Títol: «Sobre lo bello, el arte y la evolución del cerebro» Sala: Prat de la Riba
13.00- 14.45h	**Dinar**	13.00- 14.45 h	**Dinar** i sessió de **pòsters P43-85**
14.45- 16.30 h	**Sessió 2A** Comunicacions Orals **Malalties neurodegeneratives I** Sala: Prat de la Riba **Sessió 2B** Comunicacions Orals **Neurotransmissió** Sala: Pere i Joan Coromines	14.45- 16.30 h	**Sessió 4A** Comunicacions Orals **Receptors i senyalització cel.lular** Sala: Prat de la Riba **Sessió 4B** Comunicacions Orals **Cognició, comportament i trastorns mentals** Sala: Pere i Joan Coromines
16.30- 17.30 h	**Conferència plenària 2**: **Michal Schwartz** **Weizmann Institute of Science (Israel)** Títol: «Breaking immune suppression for empowering the immune system to fight against Alzheimer's disease and dementia» Sala: Prat de la Riba	16.30- 17.30 h	**3r Premi Ramon Turró** Conferència d'acceptació **Isidre Ferrer** **Universitat de Barcelona, Hospital de Bellvitge** Títol: «Naturally-occuring cell death and phagocytosis in the cerebral cortex» Sala: Prat de la Riba
17.30- 19.00 h	Sessió de **pòsters P1-42** **BEER and LIVE MUSIC amb la banda ¨*Books and Roses*¨**	17.30- 18.00 h	**Premis Millors Pòsters** Clausura

Societat Catalana de BIOLOGIA

PROGRAMA

Day 1. October 6, 2016		Day 2. October 7, 2016	
8.30- 8.45 h	Registration		
8.45 h	**Welcome presentation** Room: Prat de la Riba		
9.00- 10.45 h	**Session 1A** Oral communications **Glial cells and neuroinflammation** Room: Prat de la Riba **Session 1B** Oral communications **Neurodevelopment, neurogenesis and stem cells** Room: Pere i Joan Coromines	9.00- 10.45 h	**Session 3A** Oral communications **Neurodegenerative disorders II** Room: Prat de la Riba **Session 3B** Oral communications **Motor and sensory systems and novel therapies** Room: Pere i Joan Coromines
10.45- 12.00 h	Coffee and **poster session P1-42**	10.45- 12.00 h	Coffee and **poster session P43-85**
12.00- 13.00 h	**Plenary lecture 1**: **Rüdiger Klein** **Max-Planck Institute of Neurobiology (Germany)** Title: «Axon guidance and cues during neuronal development» Room: Prat de la Riba	12.00- 13.00 h	**Plenary lecture 3**: **Javier De Felipe** **Univ. Politècnica de Madrid, Instituto Cajal** Title: «Sobre lo bello, el arte y la evolución del cerebro» Room: Prat de la Riba
13.00- 14.45 h	**Lunch**	13.00- 14.45 h	**Lunch and poster session P43-85**
14.45- 16.30 h	**Session 2A** Oral communications **Neurodegenerative disorders I** Room: Prat de la Riba **Session 2B** Oral communications **Neurotransmission** Room: Pere i Joan Coromines	14.45- 16.30 h	**Session 4A** Oral communications **Receptors and cell signaling** Room: Prat de la Riba **Session 4B** Oral communications **Cognition, behaviour and mental disorders** Room: Pere i Joan Coromines
16.30- 17.30 h	**Plenary lecture 2**: **Michal Schwartz** **Weizmann Institute of Science (Israel)** Title: «Breaking immune suppression for empowering the immune system to fight against Alzheimer's disease and dementia» Room: Prat de la Riba	16.30- 17.30 h	**3rd Ramon Turró Award** Acceptance lecture **Isidre Ferrer** **Universitat de Barcelona, Hospital de Bellvitge** Tittle: «Naturally-occuring cell death and phagocytosis in the cerebral cortex» Room: Prat de la Riba
17.30- 19.00 h	**Poster session P1-42** **BEER and LIVE MUSIC with ¨*Books and Roses*¨ Band**	17.30- 18.00 h	**Best Posters Awards** **Closing remarks**

ORAL COMMUNICATIONS: 10 min presentation + 5 min questions

DAY 1: Thursday, October 6th 2016

8.30-8.45	**REGISTRATION**
8.45-9.00	**SYMPOSIUM PRESENTATION and WELCOME SPEECH** *Prat de la Riba room* Carles A. Saura
9.00-10.45	**ORAL COMMUNICATIONS** **Session 1A. GLIAL CELLS and NEUROINFLAMMATION** *Prat de la Riba room* **Chairman: Carme Solà (CSIC, IDIBAPS)** O-1. THE ROLE OF GLIAL IONIC HOMEOSTASIS IN GLIA-NEURON COMMUNICATION **Morey M** O-2. ZEBRAFISH MODELS TO STUDY MLC PATHOPHYSIOLOGY **Pérez-Rius C** O-3. HIGHLY MOTILE AND MIGRATING MICROGLIA AND TUMOR-ASSOCIATED MACROPHAGES DENSELY POPULATE PSEUDO-PALISADES IN GLIOBLASTOMA MULTIFORME **Saavedra-López, E** O-4. MICROGLIA CELLS FROM THE ADULT MOUSE BRAIN PHAGOCYTE NEUTROPHILS IN CULTURE **Otxoa de Amezaga A** O-5. PERIVASCULAR MACROPHAGES ATTRACT LEUKOCYTES TO THE ISCHEMIC BRAIN TISSUE **Pedragosa J** O-6. MODULATION OF ASTROCYTIC LRRC8 CHLORIDE CHANNEL BY GLIALCAM/MLC1 **Armand-Ugón M** O-7. INVESTIGATION OF LRRC8-MEDIATED VOLUME-REGULATED ANION CURRENTS IN XENOPUS OOCYTES REVEALS NOVEL PROPERTIES **Gaitán-Peñas H** **Session 1B. NEURODEVELOPMENT, NEUROGENESIS and STEM CELLS** *Pere i Joan Coromines room* **Chairman: Rüdiger Klein (Max Planck Institute of Neurobiology)** O-8. DEEP HOMOLOGY OF A GENETIC PROGRAM CONTROLLING DOPAMINERGIC NEURON DIFFERENTIATION IN NEMATODES AND MAMMALS **Remesal L**

	O-9. SEVERE BRAIN CONNECTIVITY DEFECTS IN RHOE KNOCK-OUT EMBRYOS **Marfull P** O-10. SINGLE NEURON RNA-SEQ ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED STRIATAL SUB-TYPES **Sanders P** O-11. CYCLIN D1 IN THE REGULATION OF THE INTERMEDIATE PROGENITOR CELLS RADIAL MIGRATION **Rocandio D** O-12. PRESENILIN-1 REGULATES AXON GROWTH IN HIPPOCAMPAL NEURONS **Javier-Torrent M** O-13. CALCIUM-DEPENDENT PROTEASE CALPAIN REGULATES SURVIVAL MOTOR NEURON PROTEIN IN CULTURED MOUSE MOTONEURONS **de la Fuente S**
10.45-12.00	**COFFEE BREAK and POSTERS P1-P42**
12.00-13.00	**PLENARY LECTURE** *Prat de la Riba room* **«Axon guidance and cues during neuronal development»** Rüdiger Klein (Max-Planck Institute of Neurobiology, Germany)
13.00-14.45	**LUNCH**
14.45-16.30	**ORAL COMMUNICATIONS** **Session 2A. NEURODEGENERATIVE DISORDERS-I** *Prat de la Riba room* **Chairman: Miquel Vila (Vall de Hebron Research Institute)** O-14. CHARACTERIZATION OF RTP801 KNOCKOUT MICE: IMPLICATIONS FOR CNS DEVELOPMENT AND NEURODEGENERATION **Pérez-Sisqués L** O-15. CHARACTERIZATION OF EXOSOMAL RTP801 IN PARKINSON´S DISEASE CELL MODELS **Martín-Flores N** O-16. THE PARKINSON'S DISEASE-ASSOCIATED GPR37 RECEPTOR IS AN ADENOSINE A2A RECEPTOR REPRESSOR IN MICE **Morató X** O-17. 4E-BP1 INACTIVATION CAUSES ABERRANT PROTEIN TRANSLATION IN THE STRIATUM OF HUNTINGTON'S DISEASE **Creus-Muncunill J**

	O-18. GENETIC REDUCTION OF CDK5 IN HDHQ7/Q111 MICE AMELIORATES COGNITIVE DYSFUNCTION IN HUNTINGTON'S DISEASE **Alvarez-Periel E** O-19. IN VIVO ANALYSIS OF HUMAN IPSC-DERIVED NEURONS FROM HUNTINGTON'S DISEASE PATIENTS **Miguez A** O-20. DECIPHERING HUNTINGTON'S DISEASE STRIATAL NETWORK DYNAMICS USING HIGH-SPEED HIGH-RESOLUTION CALCIUM IMAGING **Fernández-García S** **Session 2B. NEUROTRANSMISSION** *Pere i Joan Coromines room* **Chairman: Xavier Gasull (Universitat de Barcelona, IDIBAPS)** O-21. MOUSE AND ZEBRAFISH DEVELOPED DIFFERENT EVOLUTIONARY STRATEGIES TO REACH A COMMON GOAL: INCREASE SYNAPTIC MOLECULAR COMPLEXITY **Bayés A** O-22. STRUCTURAL PLASTICITY OF DENDRITIC SPINES DURING LONG-TERM SYNAPTIC POTENTIATION AND DEPRESSION REVEALED BY OPTOPHARMACOLOGICAL TOOLS **Bosch M** O-23. POSTSYNAPTIC PROTEOME OF HIPPOCAMPAL TRISYNAPTIC CIRCUIT AREAS: CA1, CA3 AND GENTATE GYRUS **Reig-Viader R** O-24. CPT1C ENHANCES AMPAR SURFACE EXPRESSION BY A DEPALMITOYLATION MECHANISM **Soto D** O-25. PYK2 IS ESSENTIAL FOR HIPPOCAMPAL SYNAPTIC PLASTICITY AND SPATIAL LEARNING AND MEMORY **Giralt A** 0-26. OPTICAL CONTROL OF ENDOGENOUS RECEPTORS AND CELLULAR EXCITABILITY USING TARGETED COVALENT PHOTOSWITCHES **Garrido-Charles, A**
16.30-17.30	**PLENARY LECTURE** *Prat de la Riba room* **« Breaking immune suppression for empowering the immune system to fight against Alzheimer's disease and dementia »** Michal Schwartz (Weizmann Institute of Science, Israel)
17.30-18.30	**BEER and POSTER SESSION P1-P42**
18.30-	**LIVE MUSIC with the band ¨*Books and Roses*¨**

DAY 2: Friday, October 7th 2016

9.00-10.45	**ORAL COMMUNICATIONS** **Session 3A. NEURODEGENERATIVE DISORDERS-II** *Prat de la Riba room* **Chairman: Maria Antonia Baltrons (IBB, Universitat Autònoma de Barcelona)** O-27. AMYLOID-BETA SOLUBLE OLIGOMERS EFFECT ON AMPA RECEPTORS: ROLE OF A-KINASE ANCHORING PROTEIN 79/150. **Miñano-Molina AJ** O-28. ANALYSIS OF SYNAPTIC-RELATED mRNAS EXPRESSION IN ALZHEIMER'S DISEASE **Siedlecki-Wullich DJ** O-29. RIP140: A NEW TARGET TO PREVENT NEURODEGENERATION IN X-LINKED ADRENOLEUKODYSTROPHY **Ranea-Robles P** O-30. ROLE OF CRTC1 IN STRUCTURAL SYNAPTIC PLASTICITY IN THE ADULT BRAIN DURING NEURODEGENERATION **Enríquez-Barreto L** O-31. SYNTHESIS AND CHARACTERIZATION OF RHDL-RAPOJ NANOPARTICLES Fernández-de Retana S O-32. DUAL THERAPEUTIC BENEFITS OF SELECTIVE-HISTONE DEACETYLASE 3 INHIBITION IN HUNTINGTON'S DISEASE MICE **Suelves N** O-33. PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE RESTORES COGNITIVE FUNCTION IN A MICE MODEL OF HUNTINGTON'S DISEASE **Cabezas-Llobet N** **Session 3B. MOTOR and SENSORY SYSTEMS and NOVEL THERAPIES** *Pere i Joan Coromines room* **Chairman: Jordi Llorens (IDIBELL, Universitat de Barcelona)** O-34. LOSS OF SYNAPSES AND CALYCEAL JUNCTIONS BETWEEN TYPE I VESTIBULAR HAIR CELLS AND AFFERENT ENDINGS ARE EARLY EVENTS DURING CHRONIC OTOTOXICITY IN RATS **Llorens J** O-35. FOREIGN BODY RESPONSE TO INTRANEURAL IMPLANTS IN RAT **De la Oliva N** O-36. US D´ONES DE XOC AL TRACTAMENT DE PUNTS GALLET MIOFASCIAL EN UN MODEL ANIMAL **Bosque M**

	O-37. NEUROREHABILITATION THERAPIES IN ISCHEMIC STROKE: AN APPROACH FROM HUMAN TO MOUSE **Gabriel-Salazar M** O-38. NEUREGULIN 1-ERBB MODULE IN C-BOUTON SYNAPSES ON SOMATIC MOTOR NEURONS: MOLECULAR COMPARTMENTATION, CHANGES DURING ALS AND RE-SPONSE TO PERIPHERAL NERVE INJURY **Salvany S** O-39. PAIN RESPONSES AND BEHAVIORAL DISTURBANCES IN CD1 MICE DURING ACUTE AND CHRONIC PHASES AFTER SPINAL CORD INJURY **Castany S**
10.45-12.00	**COFFEE BREAK and POSTERS P43-P85**
12.00-13.00	**PLENARY LECTURE** *Prat de la Riba room* **«Sobre lo bello, el arte y la evolución del cerebro»** Javier de Felipe (Universidad Politécnica de Madrid, Instituto Cajal, Madrid)
13.00-14.45	**LUNCH and POSTERS P43-P85**
14.45-16.30	**ORAL COMMUNICATIONS** **Session 4A. RECEPTORS and CELL SIGNALLING** *Prat de la Riba room* **Chairman: Cristina Malagelada (Universitat de Barcelona, IDIBAPS)** O-40. GLUA1 TRAFFICKING IS REGULATED BY MALONYL COA **Casas M** O-41. D2 RECEPTOR CROSSTALK WITH METABOTROPIC GLUTAMATE RECEPTORS REGULATES DOPAMINE SYNTHESIS IN RAT BRAIN **Abass D** O-42. COCAINE MODULATES OREXIN-CRF RECEPTOR HETEROMERS SIGNALLING IN THE VENTRAL TEGMENTAL AREA **Navarro G** O-43. LOCAL PHOTOACTIVATION OF A MGLU5 RECEPTOR ALLOSTERIC MODULATOR REDUCES PAIN IN MICE **López-Cano M** O-44. STRIATAL PHOTOACTIVATION OF AN ADENOSINE A2A RECEPTOR ANTAGONIST CAGED CONTROLS LOCOMOTION ACTIVITY IN MICE **Taura J**

O-45. NEUROMUSCULAR ACTIVITY MODULATES THE SIGNALING OF THE M2 SUB-TYPE MUSCARINIC CHOLINERGIC RECEPTOR ON PKC AND ON SNAP25 AND MUNC18-1 PHOSPHORYLATION
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O-46. QUANTITATIVE AND TIME-DEPENDENT REGULATION OF THE ALTERNATIVE SPLICING OF EXON 23A OF THE NEUROFIBROMATOSIS TYPE 1 GENE IS ESSENTIAL FOR A CORRECT NEURONAL DIFFERENTIATION PROCESS
Biayna J

Session 4B. COGNITION, BEHAVIOUR and MENTAL DISORDERS

Pere i Joan Coromines room

Chairman: Mercè Masana (Universitat de Barcelona)

O-47. CDK5-DA SIGNALING HAS A KEY ROLE IN DEPRESSIVE LIKE BEHAVIOURS MANIFESTED AT EARLY DISEASE STAGE IN HD
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O-48. PPM1F IS REGULATED BY STRESS AND ASSOCIATED WITH ANXIETY AND DEPRESSION
Wingola AP

O-49. CONTRIBUTION OF THE 14-3-3 GENE FAMILY TO AUTISM SPECTRUM DISORDER
Torrico B

O-50. A DE NOVO GRIN2B MISSENSE MUTATION CAUSING RETT-LIKE SEVERE ENCEPHALOPATHY IS ATTENUATED BY D-SERINE DIETARY SUPPLEMENT
Altafaj X

O-51. PHOSPHATASE DUSP6 REGULATES HIPPOCAMPAL ERK1/2 ACTIVATION AND LONG-TERM MEMORY
Alcon C

O-52. ABSOLUTE QUANTIFICATION OF SYNGAP C-TERMINUS VARIANTS IN MOUSE CORTEX DURING POSTNATAL DEVELOPMENT BY TARGETED PROTEOMICS
Gou G

O-53. A STUDY OF THE EFFECT OF THE DISC1 GENE IN THE VULNERABILITY FOR SCHIZOPHRENIA-SPECTRUM DISORDERS THROUGH ITS ASSOCIATION WITH NEURODEVELOPMENT MARKERS
Soler J

16.30-17.30	**3rd RAMON TURRÓ AWARD** honoring the most cited article in Neurosciences performed in Catalunya published 25 years ago (1990-91) *Prat de la Riba room* **ACCEPTANCE LECTURE** **« Naturally-occurring cell death in the cerebral cortex and phagocytosis in the cerebral cortex »** Isidre Ferrer (Hospital de Bellvitge, IDIBELL, Universitat de Barcelona)
17.30-18.00	**BEST POSTERS AWARDS** to the 3 best posters presented at the X Symposium of Neurobiology *Prat de la Riba room* **CLOSING SPEECH** Carles A. Saura

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SESSIÓ II

CONFERÈNCIES PLENÀRIES

FLRT ADHESION MOLECULES REGULATE CEREBRAL CORTEX FOLDING BY CONTROLLING NEURON MIGRATION

Rüdiger Klein

Max-Planck Institute of Neurobiology, Germany

The mechanisms underlying folding of the mammalian cerebral cortex into furrows (sulci) and ridges (gyri) are incompletely understood. Recent studies indicate that gyrus development is favored by amplification of basal progenitor cells, while the mechanisms controlling sulcus formation remain largely unknown. We have found that mutant mice with genetic deletions of FLRT1 and FLRT3 adhesion molecules develop macroscopic cortical sulci with preserved layered organization and radial glial morphology. Cortex folding in these mutants does not require progenitor cell amplification, but changes in neuron migration. Morphological analyses and computational simulations suggest that absence of FLRT1/3 reduces intercellular adhesion, promotes immature neuron migration and clustering in the cortical plate, thereby leading to sulcus formation in the normally smooth mouse neocortex. Thus, regulation of intercellular adhesion of migrating neurons is critical for cerebral cortex folding.

NOTES

BREAKING IMMUNE SUPPRESSION FOR EMPOWERING THE IMMUNE SYSTEM TO FIGHT AGAINST ALZHEIMER'S DISEASE AND DEMENTIA

Michal Schwartz

The Weizmann Institute of Science, Israel

Alzheimer's disease (AD) like may other neurodegenerative diseases, is a multi-dimensional disease involving numerous biological pathways and molecules that become deviated within the brain. Attempts have been made to address several factors that are considered hallmarks of the disease, with the vast majority of them focusing on amyloid beta (Aβ) peptides and plaque formation. Thus far, none of these approaches has resulted in a disease modifying therapy. Our findings over almost two decades show that immune system activity plays an essential role in maintaining life-long brain plasticity, and that following damage to the brain, immune cells are involved at all stages of tissue repair. Specifically, we identified the brain choroid plexus epithelium as an immunological interface needed for "healing" immune cell recruitment to sites of brain pathology. In mouse models of AD, recruitment of blood-borne monocyte-derived macrophages to sites of brain pathology is associated with a therapeutic effect. We recently pointed to peripheral immune suppression as a negative player which hamper this process, and showed that boosting peripheral immunity, by transiently breaking immune tolerance, can augment recruitment of immune regulatory cells to sites of brain pathology, and to support tissue repair and reduced inflammation. Immune checkpoints are regulatory pathways which maintain systemic immune homeostasis and tolerance. Among such checkpoints, PD-1 is expressed by immune cells and negatively regulates immune responses. PD-1 blockade is currently used as an effective immunotherapy in cancer. Using a similar approach in AD animal models, we reported that anti-PD-1 antibodies are effective in reversing cognitive loss, in removal of plaques, and in restoring brain homeostasis as determined by the inflammatory molecular profile. Such an approach is not meant to be directed against any disease-escalating factor in AD, but rather it empowers the immune system of the individual to drive the process of repair, regardless of the etiology of the disease.

NOTES

SOBRE LO BELLO, EL ARTE Y LA EVOLUCIÓN DEL CEREBRO

Javier De Felipe

Instituto Cajal (Consejo Superior de Investigaciones Científicas) y Laboratorio de Circuitos Corticales (CBT, Universidad Politécnica de Madrid), Madrid

La creatividad artística es sin duda un producto de la mente humana, pero el origen del placer intelectual que produce la observación de una obra de arte y la del artista que la crea es verdaderamente un misterio; aunque no necesitamos la belleza o la percepción estética para sobrevivir, casi todo lo que el hombre crea tiene una pincelada artística. La producción y apreciación del arte parece ser un atributo exclusivo de los humanos, una adquisición reciente de la capacidad cognitiva del género *Homo*. Entre los acontecimientos más notables acaecidos durante la evolución del cerebro humano se encuentra el aumento de su tamaño y, particularmente, el gran desarrollo y diferenciación de la corteza cerebral. Al aumentar de tamaño también aumenta el número de neuronas y de conexiones sinápticas, volviéndose cada vez más complejo. Así, parece lógico suponer que gracias al aumento de la complejidad de nuestro cerebro ha sido posible el desarrollo espectacular de las funciones cognitivas y de las habilidades artísticas. ¿Pero qué tiene de especial la neocorteza humana y en qué se diferencia de la de otras especies? ¿Lo bello es percibido sólo por los seres humanos? ¿Por qué el arte nos provoca placer mental? Como uno de los cimientos de la cultura es el aprendizaje social, es decir, el aprendizaje impulsado o influenciado por la observación o la interacción con otros individuos, cabe preguntarse de qué modo afectan la cultura y el entorno social a la apreciación del arte. En esta conferencia se abordan estas cuestiones a la luz de estudios recientes que indican que la corteza cerebral humana posee ciertas características que nos distinguen de otros mamíferos. ¿Son estas diferencias el punto de partida que dio lugar al nacimiento del arte?

NOTES

CONFERENCIA 3r PREMI RAMON TURRÓ

NATURALLY-OCCURRING CELL DEATH AND PHAGOCYTOSIS IN THE CEREBRAL CORTEX

Isidre Ferrer

Institut de Neuropatologia, Servei d'Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, CIBERNED. Universitat de Barcelona, Barcelona

The paper entitled "*Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes*" (Ferrer et al., Neuroscience 39: 451-8) was published in 1990 and described the appearance of dying cells with the morphological features of apoptosis in the somatosensory cortex and medial cortical regions, as well as in the cortical subplate of the rat. Cell death occurred during the first ten days after birth, peaking at day seven to decrease thereafter. Dying cells predominated in the future layers II and III and in the subplate and correlated with the arrival and settlement of cortical afferents at the different cortical levels, thus pointing to the likelihood that transitory cell populations participated in the modulation of the final structure of the cerebral cortex. Subplate neurons served as transient scaffolds to help the arrival of afferents in the cerebral cortex, and useless targets were eliminated once the afferents reached the appropriate sites. Amoeboid microglia populated the subplate and upper layers of the cortex and engulfed cell debris, thus being actively involved in the removal of dead cells.

The study was carried out at a time in which several researchers were engaged in trying to understand neurogenesis and gliogenesis in the brain, the development of connections including dendrites, spines and axons, and the phenomenon of transient events during corticogenesis in which cell populations and cellular processes were over-produced to be pruned shortly after to permit a definite organization of the cerebral cortex. The study paralleled research focused on dating the birthday in the periventricular germinal layer, the migration process and the definite localizations of particular neuronal populations in the cerebral cortex. By using specific markers to label DNA replication, a gradient of cell migration was established in the cerebral cortex in which neurons of the molecular layer and neurons of the subplate were the first to migrate whereas those in the cortical plate migrated following a gradient in which neurons to the inner layers were produced and migrated first followed by neurons of the middle layers and then by neurons eventually forming the upper cellular layers. Considering these aspects, postnatal cell death mainly affected the first (those of the subplate) and the latest (those of upper cellular layers) generated neuroblasts directed to the cerebral neocortex.

Amoeboid microglia, as initially described by Pio del Rio Hortega, migrated from the wall of the lateral ventricles to the future subcortical white matter, and from the pial surface to the upper cortical layers. Therefore, amoeboid microglia were phagocytes actively involved in the modeling of the cerebral cortex during development.

The paper was published at the same time as another describing the pattern of developmental cell death in the hippocampus and subiculum, and it was followed by a review linking all these development events with a focus on the rodent brain.

Research in the following years was focused on different aspects. On the one hand, efforts were made to characterize naturally-occurring cell death during development as an active process linked to the caspase-dependent apoptotic pathway; on the other hand, the concept of naturally-occurring cell death served to galvanize the idea of pathological cell death during development and

the generation of different models in the rat in which selective cell death was produced by using single doses of ionizing radiation at definite days of gestation.

Interest in naturally-occurring and induced apoptotic cell death during development extended to the study of the role of apoptosis in pathologic conditions related to hypoxia-ischemia in the infant and adult brain, and in experimental models of neurodegeneration induced by various agents. As with earlier studies geared to uncovering the pathology of human cortical malformations, these series of experimental designs were intended to learn about infarcts and mechanisms of cell death in human neurodegenerative diseases.

Several studies were centered on the study of vulnerability and cell death after global and focal ischemia in the infant and adult rat brain. Apoptosis was identified as a common cause of cell death in hypoxia-ischemia in newborn rats. Apoptosis and a mixture of apoptosis and other forms of cell death occured after global ischemia in rats and gerbils. Although necrosis was the paradigm of cell death in the core of the infarct following focal ischemia, apoptosis predominated at the periphery of the core in the so-called penumbra area. Moreover, the extent of the penumbra was susceptible to control by drugs, thus permitting reduction of the final volume of the infarction under appropriate settings.

Finally, some experiments were carried out with excitotoxic agents to gain understanding about the type of cell death in models of neurodegeneration. A complex scenario occurred in these circumstances. Although apoptosis was in fashion, our observations indicated that the type of cell death was neither apoptosis nor necrosis but rather a mixed form which was later accepted as such in subsequent experiments.

Moving to human neurodegenerative diseases, apoptosis was considered a principal cause of cell death in prevalent diseases such as Alzheimer's, Parkinson's and Huntington's, among others. However, the causes of cell death in human neurodegenerative diseases are multiple and several mechanisms converge in the degeneration and death of nerve cells. Excepting Creutzfeldt-Jakob disease, in which apoptosis was clearly demonstrated, and certain inflammatory diseases of the brain and spinal cord, we considered that the "apoptosis" described in AD and others was mostly an artifact related to the agonic state and post-mortem delay between death and tissue processing.

This summary is centered on the paper appearing in 1990 but encompasses a series of experimental studies produced during the near previous and subsequent decades. This date is also important as it is coincidental in time with the first calls of the Fondo de Investigación Sanitaria (FIS) of the Instituto de Salud Carlos III which led to the founding of projects directed at applied research in human diseases in Spain.

NOTES

SESSIÓ 1A

CEL·LULES GLIALS I NEUROINFLAMACIÓ

ORALS

O-1. THE ROLE OF GLIAL IONIC HOMEOSTASIS IN GLIA-NEURON COMMUNICATION

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The importance of glia-neuron communication during development and adulthood is becoming evident. Deregulation of this interaction can result in leukodystrofy, that is vacuolization and edema of the brain due to disruption of the myelin sheath. The CLCN2 chloride channel has been related to some types of leukodystrofy. Analysis of *CLCN2* mutant mice suggests that leukodystrophy is a result of impaired ionic glial homeostasis early in development. However, the consequences of this ionic impairment on glial function, and the secondary effects on neurons are still unknown. We turned to *Drosophila* since the intimate relationship between glia and photoreceptors early in development of the visual system allows studying the role of this channel in glia-neuron interactions. We have detected expression of the *CLCN2* Drosophila homolog gene *ClC-a* in glial cells in the developing brain. Mutant *ClC-a* animals show defects in photoreceptor axon guidance during development and RNAi depletion of *ClC-a* transcripts exclusively in glia phenocopies the guidance defects. The glia-photoreceptor interaction in the early development of the visual system is mediated by Slit/Robo signaling. Slit secretion from glial cells is necessary for the correct guidance of photoreceptors. *ClC-a* mutant glia is morphologically wild type and transcribes *slit*. Together with the genetic interaction observed between *ClC-a* and *slit*, these results suggest that ClC-a mediated glial homeostasis regulates Slit secretion. Our hypothesis is that impairment of ionic homeostasis in glia might be causing defects in secretion of molecules important in glia-neuron communication events.

NOTES

O-2. ZEBRAFISH MODELS TO STUDY MLC PATHOPHYSIOLOGY

Pérez-Rius C[1], Folgueira M[2], Sirisi S[1,3],Gaitán-Peñas H[1,4], Alia A[5,6], Estévez R[1,4] and Barrallo-Gimeno A[1,4]

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare type of leukodystrophy characterized by myelin vacuolization. MLC is either caused by mutations in the astrocytic protein MLC1 or the cell adhesion molecule GLIALCAM. Both proteins interact in vivo in astrocyte-astrocyte junctions. Furthermore, GlialCAM is also expressed in oligodendrocytes and has been showed to be an auxiliary subunit of the chloride channel ClC-2 in glial cells. Histological studies performed in *Mlc1*$^{-/-}$ and *GlialCAM*$^{-/-}$ mice showed that the expression and appropiate subcellular localization of each protein was mandatory for the appropiate targeting of the other. Furthermore, ClC-2 has also been involved in MLC pathogenesis due to its mislocalization in both *Mlc1*$^{-/-}$ and *GlialCAM*$^{-/-}$ mice, suggesting an essential role in brain ion homeostasis. However, Mlc1 and GlialCAM are not affected by the lack of ClC-2, as evidenced by experiments performed in *Clcn2*$^{-/-}$ mice. Our aim is to use the zebrafish as an alternative model to study MLC disease. By means of sequence database search, we found a single ortholog for *MLC1* and two *glialcam* paralogues due to the teleost-specific genome duplication after its divergence from the tetrapods: *glialcama* and *glialcamb*. We decided to focus only on glialcama as it was able to target both mlc1 and rat ClC-2 to cell junctions *in vitro*, whereas glialcamb presented a diffuse location. Surprisingly, we found out that *CLCN2* suffered an additional single gene duplication after the genome duplication, hence, three paralogs exist: *clcn2a*, *clcn2b* and *clcn2c*. clc-2a and clc-2b could be considered the homologs to mammalian ClC-2 in terms of expression pattern and electrophysiological characteristics. We decided to focus specially on clc-2a as it showed stronger expression in the brain and eye. In this symposium we will present our progress in obtaining *mlc1*$^{-/-}$, *glialcama*$^{-/-}$ and *clcn2a*$^{-/-}$ zebrafish and the studies performed so far on these models.

NOTES

O-3. HIGHLY MOTILE AND MIGRATING MICROGLIA AND TUMOR-ASSOCIATED MACROPHAGES DENSELY POPULATE PSEUDO-PALISADES IN GLIOBLASTOMA MULTIFORME

Saavedra-López, E.[1], Cribaro, G.P.[1], Casanova, P.V.[1], Pérez-Vallés, A.[2], Gallego-Sánchez, J.M.[2], Barcia, Sr. C.[2], Barcia, Jr. C[1]

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Gliomas are the most common type of primary brain tumours, they present a very grim prognosis and currently remain incurable. Glioblastoma multiforme (GBM) is the highest-grade astrocytoma and presents a series of histopathological hallmarks, namely glomeruloid bodies, necrotic areas, gemistocytic formations, aberrant mitoses and pseudo-palisades. Precisely, these pseudo-palisades are poorly understood, although they are increasingly often regarded and they are thought to be key structures in the propagation of the tumor. Reports hypothesize that when a blood vessel collapses, tumour cells escape the harsh environment created by the lack of nutrients such as glucose or oxygen, therefore creating this palisade-like structure. Moreover, references state that the inflammatory component in these structures is very low.

We, however, have found by studying pseudo-palisades in six human GBM samples, that these regions have a vast amount of tumor-associated microglia/macrophages (TAM/Ms). In addition, when quantifying these cells we saw that their density does not depend on the tumours' aggressiveness, meaning that they are always present in a similar degree. Furthermore, by means of confocal microscopy, we observed evidence of TAM/Ms directionality within these structures, demonstrating their motility. Finally, in an *in vitro* model, we show with time-lapse imaging that TAM/Ms respond with high motility in comparison to glioma cells to the conditions found in pseudo-palisades. This finding sheds light on the understanding of GBM microenvironments, infers the versatility of TAM/Ms and their quick and highly-motile response, bringing the possibility of manipulate TAM/Ms to eradicate tumor cells, elucidating new therapies against this fatal disease.

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NOTES

O-4 . MICROGLIA CELLS FROM THE ADULT MOUSE BRAIN PHAGOCYTE NEUTROPHILS IN CULTURE

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Ischemic stroke causes an acute inflammatory response involving microglia activation, the brain resident immune competent cells. Following activation, microglia retract their branching processes, migrate to the site of injury, and clear cellular debris by phagocytosis. For these important functions, it is crucial to understand microglial phagocytosis in health and disease. In ischemic mice, our studies with confocal microscopy suggest that these cells are able to phagocyte infiltrating neutrophils, in agreement with recent findings (Newmann et al., 2016)[1].

To investigate this possibility in living cells, we set an *in vitro* system where cultured microglia were exposed to neutrophils and investigated cellular interactions by time-lapse confocal microscopy. We used an immunomagnetic method to isolate microglia from the adult mouse brain and the cells were cultured for seven days. At this point, neutrophils extracted from the bone marrow of fluorescent DsRed reporter mice were added to the microglia cultures. Here we report the results of treatment with the purinergic $P2Y_{12}$ receptor antagonist PSB0937.

Adhered microglia cells were frequently extending/retracting processes, whereas neutrophils moved above microglia. They seemed to scan the microglia surface. Measurements with Image J Software in the registered videos (14 hours) showed that about 20% of the neutrophils were engulfed and degraded by microglia. PSB0937 treatment reduced by 30% the numbers of phagocyted neutrophils, suggesting that P2Y12 regulates microglial activity. We also observed NETosis in 28% of total neutrophils, involving the apparent restructuration of the nucleus, clear expulsion of the intracellular content and the increase in cell size. PSB0937 treatment soared up to 40% the incidence of NETosis. Nevertheless, NETosis was observed regardless of the presence of microglia. The results show that adult microglia phagocyte neutrophils in culture and suggest the involvement of purinergic receptors in this process.

[1] Neumann et al Acta Neuropathol, 2015:129:259–77

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NOTES

O-5. PERIVASCULAR MACROPHAGES ATTRACT LEUKOCYTES TO THE ISCHEMIC BRAIN TISSUE

Pedragosa J[1,2], Salas-Pérdomo A[1,2], Gallizioli M[1], Cugota R[1], Miró-Mur F[2], Pérez-Asensio F[1] and Planas AM[1,2]

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Objective: Brain perivascular and meningeal macrophages (PVMM) are brain resident macrophages located at the interface between the nervous and the immune system, and have been attributed functions of immunological surveillance. However, little is known about the role of PVMM in brain diseases. The aim of this study was to investigate the response of PVMM to ischemic stroke.

Material and Methods: Transient middle cerebral artery occlusion (tMCAo) was induced with the intraluminal technique in adult male Sprague-Dawley rats. PVMM were detected with anti-CD163 antibodies PVMM were isolated from the brain 14h post-ischemia in order to identify ischemia-induced changes in the gene expression profile of these cells using microarrays and RT-qPCR. In further experiments, PVMM where depleted by intracerebroventricular administration of clodronate liposomes (CL). tMCAo was carried out 4 days after administration of CL or vehicle liposomes (VL). 24h after tMCAO, the numbers of neutrophils, lymphocytes and macrophages in the ischemic and non-ischemic brain tissue were counted either using stereological microscopy or by flow cytometry. Infarct volume and the neurological deficits were assessed 24h post-ischemia.

Results:. Ischemia induced marked changes in the PVMM gene expression profile involving alterations in various biological processes. Notably, it increased the PVMM mRNA expression of factors involved in leukocyte chemotaxis. CL caused a dramatic reduction of PVMM without affecting microglia cell number. Following ischemia, rats devoid of PVMM showed significantly less numbers of infiltrating neutrophils and CD68+ macrophages but higher numbers of CD3+ lymphocytes in the ischemic tissue suggesting that PVMM regulated leukocyte recruitment to the injured brain tissue. PVMM depletion did not significantly change the neurological deficits and the brain lesion 24h after tMCAo.

Conclusion: This study supports the involvement of PVMM in regulating leukocyte infiltration to the brain lesion. Any potential contribution of PVMM to stroke outcome needs evaluation at time points beyond 24h.

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NOTES

O-6. MODULATION OF ASTROCYTIC LRRC8 CHLORIDE CHANNEL BY GLIALCAM/MLC1

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare type of leukodystrophy caused by mutations in MLC1 and/or GLIALCAM. Lack of these proteins cause water accumulation leading to myelin vacuoles. It has been shown that volume-regulated chloride currents, formed by heteromers of LRRC8 proteins, are defective in MLC. In this work, we adressed the mechanism of functional regulation of LRRC8 proteins by MLC1. We could demonstrate that these proteins are functionally linked, although in an indirect manner. MLC1 and LRRC8 do not interact physicially, nor do colocalize in astrocyte junctions. By proteomic analyses of immunoprecipitated LRRC8 proteins, we find that lack of MLC1 influence LRRC8 subunit composition and posttranslational modifications of some LRRC8 subunits. We are beginning to understand the role of GlialCAM/MLC1 in astrocyte and myelin homeostasis.

NOTES

O-7. INVESTIGATION OF LRRC8-MEDIATED VOLUME-REGULATED ANION CURRENTS IN XENOPUS OOCYTES REVEALS NOVEL PROPERTIES

Gaitán-Peñas H[1,2], Gradogna A[3], Laparra-Cuervo L[4] , Solsona C[5] , Fernández-Dueñas V[6] , Barrallo-Gimeno A[1,2] , Ciruela F[6], Lakadamyali M[4], Pusch M[3], Estévez R[1,2]

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare inherited disease characterised by chronic white matter oedema. Two disease causing genes have been identified: MLC1 and HEPACAM. It has been shown that knock out and knock down astrocytes lacking the proteins MLC1 or glialCAM (encoded by HEPACAM), exhibit vacuolation and reduced volume-regulated anion-channel (VRAC) currents. VRAC channels play an important role in controlling cell volume by opening upon cell swelling. Recent work has shown that heteromers of LRRC8A with other LRRC8 members (B, C, D, E) form the VRAC channel. Using *Xenopus* oocytes we have developed a simple system to study LRRC8 proteins. We discovered that the addition of fluorescent proteins to the C-terminus of the LRRC8 proteins resulted in constitutive anion channel activity. Taking advantage of these approach, we were able to reproduce previous findings indicating that LRRC8 heteromers mediate anion and osmolyte flux with subunit-dependent kinetics and selectivity. Additionally, we found that LRRC8 heteromers mediate glutamate and ATP flux, and that the inhibitor carbenoxolone acts from the extracellular side, binding to probably more than one site. Our results also suggest that the stoichiometry of LRRC8 heteromers is variable, with a number of subunits ≥6, and that the heteromer composition depends on the relative expression of different subunits. The system described here enables easy structure-function analysis of LRRC8 proteins. Furthermore, with the information obtained from proteomic analysis, our next purpose is to study the link between the proteins MLC1/glialCAM and the LRRC8's mediated VRAC activity.

NOTES

SESSIÓ 1B

DESENVOLUPAMENT, NEUROGÈNESI I CÈL.LULES MARE

ORALS

O-8. DEEP HOMOLOGY OF A GENETIC PROGRAM CONTROLLING DOPAMINERGIC NEURON DIFFERENTIATION IN NEMATODES AND MAMMALS

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Dopamine (DA) is one of the main neurotransmitters in the brain and regulates a variety of complex behaviors. Understanding the regulatory logic of DA terminal differentiation is important for both basic as well as translational research. All DA neurons, from any organism, express a battery of phylogenetically conserved genes involved in the synthesis, release and re-uptake of dopamine, termed "DA pathway genes". Using the model organism C. elegans our lab has found that a combination of three transcription factors (TFs) from three different families (ETS, DLL and PBX) directly regulate DA pathway genes expression.

In mammals, olfactory bulb dopaminergic neurons are the more ancestral in evolution. Interestingly mouse orthologs for both ETS (Etv1/Er81) and DLL (Dlx2) TFs are already known to be required for mouse olfactory bulb (OB) DA differentiation. Pbx1 and Pbx2 have been recently shown to control olfactory bulb neurogenesis, however a specific role on dopaminergic terminal differentiation has not been assessed so far.

Here we show that Pbx1 is expressed in the adult OB DA neurons and specific DA lineage conditional Pbx1 mutants mice exhibit a dramatically decrease in the number of DA neurons of the OB. In addition, ectopic expression of Pbx1 in the mouse OB is sufficient to induce DA differentiation. To further understand the functional consequences of the loss of DA neurons we have performed some behavioral assays and the preliminary results also indicate olfaction defects in the Pbx1 mutants. Considering the evolutionary conservation of PBX, ETS and DLX TF on dopaminergic neuron, we further analyzed additional factors required for mouse DA specification and surprisingly we found that their worm ortholog is also required in nematode DA specification. Our findings demonstrated a remarkable extent of homology in the specification of a critically important neuronal cell type, conserved over a billion years of evolution.

NOTES

O-9. SEVERE BRAIN CONNECTIVITY DEFECTS IN RHOE KNOCK-OUT EMBRYOS

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Axon guidance regulation in the developing brain requires molecular cues that act through specific receptors to remodel axon cytoskeleton and trigger attractive or repulsive responses. In thalamocortical axons (TCAs), migration of the so-called "corridor cells" is necessary for TCAs to cross the subpallium. Later in development, corticofugal axons have been proposed to help TCAs to reach the pallium. Other axon-axon interactions, such as the striatal-thalamic interaxonal interactions have been proposed to help TCAs. Rho GTPases are well-known transducers of the effect of axon guidance cues on cytoskeleton dynamics. RhoE is an atypical RhoGTPase involved in axon growth and neuron migration. RhoE knock-out mice (RhoE gene-trapping allele or gt) show postnatal lethality, neurodevelopmental delay and impairment of olfactory bulb development. We used this mouse model to study brain axonal connectivity during development. Immunofluorescence and DiI/DiA tracing analysis revealed that RhoEgt/gt mice show severe axonal projection defects: TCAs are unable to cross the diencephalon-telencephalon boundary (DTB) and striatonigral axons (SNAs) are misguided ventrally. Surprisingly, Islet1 staining shows a properly formed corridor at rostral levels, although it is wider at caudal ones. We propose that TCAs missdevelopment is indeed secondary to SNAs misguidance for which RhoE is a key signaling regulator. We are currently working on the hypothesis that SNAs exert an attractive/permissive effect on TCAs to cross the DTB. In summary, our results indicate a function of RhoE in the correct development of brain connectivity and an important role of SNAs in TCAs guiding through the DTB.

NOTES

O-10. SINGLE NEURON RNA-SEQ ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED STRIATAL SUB-TYPES

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The development and characterisation of robust and efficient *in vitro* differentiation protocols for the generation of functional striatal neurons from human pluripotent stem cells (hPSCs) is essential for both the study of striatal development and the molecular and cellular mechanisms involved in Huntington's disease (HD). Furthermore, such protocols are required for the development of transplantation based cell therapy treatment of HD patients.

We utilise such a differentiation protocol, with transcriptional, immunocytochemical and physiological characterisation revealing that functional striatal neurons are generated from hPSCs within 37 days *in vitro* (DIV). However, further characterisation is required to identify the specific striatal neuron sub-types that are present.

Given that few good antibodies are available against the known striatal neuron sub-type markers and that a population level analysis of gene expression is insufficient, transcriptomic analysis at the single cell level is required to identify the neuronal sub-types that are present. To achieve this we isolated single neurons from hPSC-derived neuronal networks at DIV 21 and 37. Using flow cytometry these single neurons were sorted into 384 well plates and their transcriptomes were sequenced using massively parallel RNA single-cell sequencing (MARS-seq).

Transcriptomic profiles were obtained from a high percentage of the isolated neurons with RNA types identified including protein coding RNA, mitochondrial RNA and long non-coding RNA. Identification of neuronal sub-types is currently underway in both a directed manner, based on the expression of known markers, and using methods such as unbiased hierarchical clustering, principal component analysis and t-distributed stochastic neighbour embedding to identify neuronal sub-types in an unbiased manner.

The data we are generating will identify the striatal neuron sub-types present in our cultures. Furthermore, we anticipate that this approach will identify novel neuronal sub-type markers.

This study was supported by grants from the Ministerio de Economia y Competitividad and from the ISCIII-Subdirección General de Evaluación and European Regional Development Fund (ERDF) [RETICS and CIBERNED], Spain; and CHDI Foundation, USA.

NOTES

O-11. CYCLIN D1 IN THE REGULATION OF THE INTERMEDIATE PROGENITOR CELLS RADIAL MIGRATION

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The best known function of CyclinD1 within the cell is the integration of extracellular signals to cell division. Recent studies show that CyclinD1 may be located as well in the cytoplasm where it has been proposed to regulate cell adhesion in macrophages or keratinocytes. Nervous system development is characterized by several processes such as migration, polarization and differentiation that require a thigh regulation of cell adhesion. In this context, we wanted to address the role of the cytoplasmic CyclinD1 in vivo. Intriguingly, we observed that CyclinD1 was expressed in the radial glial process of some populations of radial glial cells besides its characteristic expression in the nucleus of the progenitor cells in the proliferative ventricular zone of the telencephalon. Interestingly, this cytoplasmic localization of CyclinD1 follows a spatiotemporal pattern paralleling the initiation of neurogenesis and radial glial directed neuronal migration. These results suggest a possible role of CyclinD1 unrelated to cell proliferation, perhaps in the regulation of cell adhesion as previously suggested during keratinocyte differentiation. The analysis of CyclinD1 deficient mice reveals an abnormal pattern of Tbr2 staining where more positive cells are located in the intermediate zone, suggesting a adhesion role of cytoplasmic CyclinD1 in the radial migration of postmitotic subventricular progenitors through radial glial cells.

NOTES

O-12. PRESENILIN-1 REGULATES AXON GROWTH IN HIPPOCAMPAL NEURONS

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Presenilin-1 (PS1), the catalytic component of γ-secretase that regulates the processing of multiple transmembrane proteins, is mutated in the majority of cases of familiar Alzheimer's disease (AD). Recent evidences indicate that PS1 plays a critical role during brain development, although the molecular mechanisms involved are largely unknown. We found that loss of PS1/γ-secretase function during development leads to brain abnormalities in $PS1^{-/-}$ embryos. Specifically, $PS1^{-/-}$ embryos show brain hemorrhages and reduction of axon length in neural progenitor cells in the hippocampus and ventricular zone, as revealed by immunofluorescence and quantitative imaging analyses of axonal markers. Similar reduction of axon length is observed in cultured hippocampal neurons from $PS1^{-/-}$ embryos and this reduction can be mimicked by using alpha/γ-secretase inhibitors. We found accumulation of PS1/γ-secretase substrates in PS1-deficient neurons, and that the effect of PS1 on axon elongation is mediated by RhoA signalling since a RhoA inhibitor and a dominant-negative mutant efficiently reversed axon defects caused by PS/γ-secretase deficiency. Since the family of ephrin receptors (Eph) have important roles in axon guidance and migration, we analyzed by quantitative real-time RT-PCR the expression levels of EphA receptors in primary hippocampal neurons at distinct differentiation stages. Interestingly, our results indicate that EphA receptors are differentially expressed dependently on the presence of PS1 during neuronal development in cultured hippocampal neuron. In summary, our results suggest that PS1/γ-secretase plays an essential role on axon growth in developing neurons and that multiple mechanisms may account for this effect.

This work was funded by grant from the Spanish Ministerio de Economía y competitividad (SAF2013-43900-R and CIBERNED CB06/05/0042) and Generalitat de Catalunya (2014 SGR0984).

NOTES

O-13. CALCIUM-DEPENDENT PROTEASE CALPAIN REGULATES SURVIVAL MOTOR NEURON PROTEIN IN CULTURED MOUSE MOTONEURONS

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Muscular Atrophy (SMA) is a genetic disease that causes the selective degeneration of spinal cord motoneurons (MNs). It is originated by the deletion or mutation of Survival Motor Neuron 1 gene and the reduction of Survival Motor Neuron (SMN) protein level. Because SMN levels are crucial for the disease progress SMA therapeutics development is focused on the identification of compounds that increase SMN protein. Several strategies have been developed to increase SMN; however, nowadays no treatment is available.

SMN protein stability and degradation have been largely unexplored, but it is known that SMN can be processed in vitro by Calpain, a calcium-dependent protease implicated in muscle disorders and neurodegenerative diseases. The purpose of the present study is to analyse the effect of Calpain reduction or Calpain activation in SMN level in cultured spinal cord MNs. Calpain reduction was performed by a Calpain specific shRNA. After lentiviral transduction of shRNA, SMN protein levels were analysed by western blot. Results show an increase of SMN level in Calpain knockdown conditions. Calpain activation was induced by adding potassium into the culture medium. Calpain activation was monitored by alpha-spectrin processing fragments, and SMN levels were determined. The results demonstrate that Calpain activity reduce SMN protein level.

Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias (PI14/0060), Unión Europea, Fondo Europeo de Desarrollo Regional (FEDER), "Una manera de hacer Europa"; Comissionat d'Universitats i Recerca, Departament d'Innovació, Universitats i Empresa de la Generalitat de Catalunya i Fons Social Europeu; Universitat de Lleida (Programa d'Ajuts Predoctorals).

NOTES

SESSIÓ 2A

MALALTIES NEURODEGENERATIVES I

ORALS

O-14. CHARACTERIZATION OF RTP801 KNOCKOUT MICE: IMPLICATIONS FOR CNS DEVELOPMENT AND NEURODEGENERATION

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RTP801 is a stress-regulated protein with a crucial role inhibiting mTOR/Akt signaling pathway via the tuberous sclerosis complex (TSC1/2). Many processes have been described to be related with mTOR signaling in the nervous system, such as proliferation, survival, axon sprouting, memory and plasticity. In addition to its regulatory role in cortical development and peripheral nervous system myelination, RTP801 has been studied in neurological diseases, where it has a pro-apoptotic function. RTP801 knockout mice have been useful to better understand RTP801 relevance in the central nervous system, but these animals have never been thoroughly characterized. Here in this work we first studied RTP801 knockout mice at a behavioral, histological and biochemical level. We found that RTP801 is important for proper motor function since its absence causes notable motor impairment together with hind-limb clasping phenotype, which is tightly related to neurodegeneration. Remarkably, knockout animals display an aberrant distribution of cells in the cortex and widespread reactive astrogliosis. Moreover, TrkB and p75NTR neurotrophic receptors protein levels appear altered, suggesting a role of RTP801 in BDNF signaling. Altogether, behavioral and histological data reveal that RTP801 has an important role in CNS development and motor function and the biochemical analyses point towards a possible novel role of RTP801 in BDNF-induced mTOR signaling.

NOTES

O-15. CHARACTERIZATION OF EXOSOMAL RTP801 IN PARKINSON´S DISEASE CELL MODELS

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RTP801 is a cell stress responsive protein, which expression is increased in samples from patients suffering from neurodegenerative diseases. RTP801 protein is increased in dopaminergic neurons in sporadic and mutant parkin Parkinson's disease (PD) patients, in the caudate and cerebellum of Huntington's disease (HD) patients, and in lymphocytes from Alzheimer's disease affected subjects. Its over expression is sufficient to promote neuronal death in cellular models of PD by a mechanism involving a sequential inhibition of mTOR and Akt kinases. In HD, RTP801 also mediates mutant huntingtin neuronal toxicity.

Interestingly, RTP801 can aggregate in the cytoplasm of cells, and aggregation of proteins is recognized as a prominent feature of many neurodegenerative disorders. Although is poorly understood, exosomes play an important role as carriers of biological material from cell to cell, and their function as conveyors of cellular components has been linked to the progression of neurodegeneration.

Here we investigated whether RTP801 can be found in exosomes and whether this process is sensitive to PD mimetic 6-hydroxydopamine (6-OHDA).

Our results indicate that both ectopic and endogenous RTP801 are found in the exosomal fractions isolated from culture media from both HEK293 cells and rat cortical neurons. Interestingly, 6-OHDA elevated both intracellular and exosomal RTP801 protein levels. Furthermore exosomes isolated from 6-OHDA-exposed neurons were more toxic on the recipient cells than exosomes from control neuronal cultures.

All together, these findings indicate that RTP801 is present in exosomal vesicles under a pathological context and that it could participate in the trans-neuronal communication in neurodegeneration.

NOTES

O-16. THE PARKINSON'S DISEASE-ASSOCIATED GPR37 RECEPTOR IS AN ADENOSINE A2A RECEPTOR REPRESSOR IN MICE

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GPR37, also known as parkin-associated endothelin-like receptor (Pael-R), is an orphan G protein-coupled receptor that has been related to the Parkinson disease (PD) neuropathology. Interestingly, it has been described that the genetic blockade of GPR37 enhanced the striatal dopamine transporter (DAT) cell surface expression, thus resulting in reduced dopamine (DA) content in the striatum. In addition, knocking-out GPR37 triggered anxiolytic-like effects and sensitized mice to hippocampal A2AR-mediated signalling.

Here we report that GPR37 and A2AR physically and functionally interact both in living cells and in native tissue. Thus, by using biochemical techniques (i.e. co-immunoprecipitation and proximity ligation assay) we demonstrate a physical interaction between these two receptors in the striatum. Also, immunogold detection reveals a close proximity at the postsynaptic level of striatal synapses. Interestingly, GPR37 deletion promotes striatal A2AR cell surface expression which correlates well with an increased A2AR agonist-mediate cAMP accumulation both in primary striatal neurons and synaptosomes from striatum. Furthermore, GPR37-KO mice show enhanced catalepsy induced by A2AR agonist and an increased response to A2AR antagonist-mediated locomotor activity. Overall, these results demonstrate for the first time an important role of GPR37 controlling A2AR cell surface targeting and functionallity.

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NOTES

O-17. 4E-BP1 INACTIVATION CAUSES ABERRANT PROTEIN TRANSLATION IN THE STRIATUM OF HUNTINGTON'S DISEASE

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Protein synthesis is a fundamental process in all cells but, when exaggerated, it is proposed to be the cause of different neurodegenerative and neurological disorders such as Parkinson's disease, fragile X syndrome or autism. Huntington's disease (HD) is a neurodegenerative disease caused by a CAG repeat expansion in the exon-1 of the huntingtin gene, generating a mutant huntingtin (mhtt) protein. Although the expression of mhtt is ubiquitous, striatal projection neurons are the main affected population. The molecular mechanisms that account for this specific vulnerability are not fully understood. In HD, there is an alteration in the levels and/or activity of several kinases, some of them involved in the regulation of protein synthesis. Therefore, we sought to analyze whether protein synthesis is altered in HD. For that, we focused on the study of 4E-BP1, a binding protein that inhibits cap-dependent translation by binding to eIF4E. When hypophosphorylated 4E-BP1 is functional and impedes the interaction of eIF4E with eIF4G so blocking the eIF4F complex formation. When hyperphosphorylated 4E-BP1 looses its afinity for eIF4E and protein translation is enhanced. Thus, we analyzed the phosphorylation status of 4E-BP1 in the striatum of different HD mouse models and patients. We found that 4E-BP1 is inactivated in HD striatum, correlating with an increased formation of the eIF4F complex. Accordingly, we detected aberrant de novo protein translation as assessed by the SUnSET method. Interestingly, genetic and pharmacological normalization of protein synthesis in R6/1 mice, a transgenic model of HD, prevents motor learning deficits. In conclusion, our results indicate that dysregulation of protein synthesis caused by 4E-BP1 inactivation could be a key event in HD pathogenesis, which opens new therapeutic possibilities.

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O-18. GENETIC REDUCTION OF CDK5 IN HDH$^{Q7/Q111}$ MICE AMELIORATES COGNITIVE DYSFUNCTION IN HUNTINGTON'S DISEASE

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Cognitive impairment is an early feature of Huntington's disease (HD) with increasing relevance over the classical motor symptoms. Unfortunately, molecular mechanisms underlying these defects remain unclear. Cdk5 is a serine/threonine kinase whose activity is primarily restricted to the nervous system. In recent years, Cdk5 has emerged as a key regulator of synaptic plasticity and cognition and it has been involved in many neurodegenerative diseases such as Huntington, Alzheimer or Parkinson´s Disease. Importantly, our group has demonstrated aberrant Cdk5 activity in the striatum of different HD models and in HD human brain. This data highlights Cdk5 as an important modulator of neuronal dysfunction and points out therapeutic strategies aimed to normalize Cdk5 activity as prospect means to delay or prevent HD progression. To determine whether altered Cdk5 activity could contribute to cognitive decline in HD we generated a new transgenic mouse model expressing mutant huntingtin and heterozygous for Cdk5 (Hdh$^{Q7/Q111}$; Cdk5$^{+/-}$). The genetic modulation of Cdk5 levels in Hdh$^{Q7/Q111}$ mutant mice restored corticostriatal learning deficits and long-term memory impairments. Moreover, our data shows that restoration of cognitive functions is paralleled by a recovery of NR2B surface levels in the striatum and cortex of double mutant mice. This recovery correlates with a restoration of total phospho-NR2B (Tyr1472) and phospho-Src (Tyr416) levels in the cortical region, suggesting that Cdk5 might be regulating NR2B surface levels through this pathway. Altogether, these findings demonstrate that modulation of Cdk5 activity or signalling in HD may contribute to restore synaptic plasticity and learning deficits in this devastating disorder.

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NOTES

O-19. IN VIVO ANALYSIS OF HUMAN IPSC-DERIVED NEURONS FROM HUNTINGTON'S DISEASE PATIENTS

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Huntington's disease (HD) is a hereditary neurodegenerative disorder mainly characterized by striatal atrophy and degeneration of medium spiny neurons (MSNs). Although mouse models have provided a substantial amount of information about HD, they show important limitations for understanding the pathogenesis in humans. Generation of human induced pluripotent stem cells (iPSCs) is one of the most promising technologies for the study of HD. Current *in vitro* HD iPSC models can recapitulate some disease phenotypes, but they are not useful for studying long-term differentiation, aging and the establishment of brain connections. Interestingly, new *in vivo* models using HD patient-derived iPSCs transplanted into mice could avoid these shortcomings, by allowing cell differentiation and aging within a physiologically relevant environment. Previous transplantation studies in the HD field have been hampered by the poor survival and functional integration of differentiated grafted human cells in murine models. To circumvent these drawbacks, we have transplanted iPSC-derived telencephalic progenitors from healthy subjects and HD patients transduced with a GFP lentivirus into the forebrain of neonatal mice. At this early age, patterning cues present in the host developing brain act to instruct specific cell fates and play a key role in determining the migration, connectivity and functional integration of engrafted cells. Moreover, the immature immune system of neonatal mice reduces the risk of rejection of exogenous cells, avoiding the use of immunosuppressive treatments. Our preliminary experiments show that the vast majority of transplanted cells express Ctip2, including a subpopulation co-expressing DARPP-32, indicative of MSN identity. Furthermore, cells grafted in the striatum send axons towards the globus pallidus, a well-known target of MSNs, suggesting that human iPSC-derived differentiated neurons can establish axonal projections within the basal ganglia circuitry. Remarkably, transplanted cells survived up to 5 months, allowing the examination of different HD-associated phenotypes.

This work has been supported by Ministerio de Economía y Competitividad, Spain; ISCIII-Subdirección General de Evaluación and European Regional Development Fund (ERDF) [RETICS and CIBERNED], Spain; CHDI Foundation Inc., USA.

NOTES

O-20. DECIPHERING HUNTINGTON'S DISEASE STRIATAL NETWORK DYNAMICS USING HIGH-SPEED HIGH-RESOLUTION CALCIUM IMAGING

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG expansion in the huntingtin (htt) gene. HD pathology is most prominent in the striatum, where mutant-htt (mHtt) triggers the imbalance between the excitation/inhibition of basal ganglia output pathways, and thus, induces motor symptoms. Albeit late stages of the disease involve cell death of the medium-sized spiny neurons (MSN), functional alterations in the striatal network dynamics appear much earlier, however, they are poorly understood. The analysis of spontaneous activity in cultures of dissociated neurons could be a valuable tool to understand the impact of the mHtt in the behavior of neuronal networks. Our aim is to identify how mHtt alters the activity of the different striatal cell types in a mouse primary culture and their specific impact to the global neuronal network signature in HD. Using high-speed calcium imaging we have recorded simultaneously the spontaneous activity of hundreds of neurons from striatal cultures from WT and the R6/1 mice (DIV 10-15-21). We resolved and characterized the spontaneous activity of all single cells in the culture -by analysing the calcium-induced Fluo4 fluorescence changes. Both WT and R6/1 striatal primary cultures display spontaneous activity. At the single cell level, we identified different populations based on their fluorescence activity traces through supervised learning algorithms (machine learning), and obtained precise firing patterns. At the population level, the average activity pattern was altered in R6/1 cultures, indicating a functional alteration in HD striatal network dynamics. Preliminary data indicates that striatal disinhibition is also impaired by mHtt, as R6/1 cultures showed altered response to GABA receptor antagonism (bicuculline). Moreover, we are currently characterizing the contribution of neurons and glia to the striatal activity pattern. Understanding functional network alterations in HD is fundamental to decipher initial key mechanisms to finally target early symptoms.

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NOTES

SESSIÓ 2B

NEUROTRANSMISSIÓ

ORAL

O-21. MOUSE AND ZEBRAFISH DEVELOPED DIFFERENT EVOLUTIONARY STRATEGIES TO REACH A COMMON GOAL: INCREASE SYNAPTIC MOLECULAR COMPLEXITY

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Mammalian synapse proteomes have been studied intensively, so that we have a fairly complete catalogue of proteins involved in mammalian synaptic function. Yet, very little is known about their composition in other vertebrate species, hindering our understanding of its evolution. Here we report the first large-scale characterisation of the zebrafish synaptic proteome and compare it with that of mouse. Special interest has been placed in analysing how the teleost-specific whole genome duplication has contributed to the synaptic proteome of present day fish. Importantly, we found that the complexity of the zebrafish postsynaptic density proteome is lower than in mammals. Having fewer proteins and fewer protein families. Instead, zebrafish displayed larger protein families, arising from the teleost whole genome duplication. Furthermore, our data indicates that synaptic genes arising from this whole genome duplication have been retained in the genome at higher rates than other genes expressed in the brain, indicating they neo/sub-functionalised acquiring important functions for animal fitness.

NOTES

O-22. STRUCTURAL PLASTICITY OF DENDRITIC SPINES DURING LONG-TERM SYNAPTIC POTENTIATION AND DEPRESSION REVEALED BY OPTOPHARMACOLOGICAL TOOLS

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The molecular mechanisms of learning and memory are based on the ability of synapses to persistently modify their properties in response to neuronal activity. These modifications can be functional and/or structural, and occur in a selective and independent manner in each individual synapse. The use of optopharmacology (light-regulated drugs) permits the selective manipulation of single neurons and single synapses with extremely high spatial and temporal resolution, therefore, being the ideal tool to study the molecular mechanisms of synaptic plasticity. Using light-activated agonists and antagonist of glutamate receptors, freely diffusible or covalently bound, we revealed how dendritic spines modify their morphology and their internal substructures during induction of long-term potentiation (LTP) and depression (LTD). We found that the postsynaptic density modifies its morphology in an asynchronous way with respect to the whole dendritic spine during LTP. On the other side, spine structural plasticity only occurs when LTD is induced through activation of NMDA receptors but not of metabotropic glutamate receptors (mGluR), although synapses are functionally depressed and AMPA receptors are internalized in both cases. We confirmed this finding at the level of single spines by using a novel mGluR-dependent LTD induction technique using a photoswitchable molecule. These results demonstrate that functional and structural plasticity of synapses and their internal components can be regulated independently, thus providing an additional layer of complexity to the molecular mechanisms that underlie learning and memory.

NOTES

O-23. POSTSYNAPTIC PROTEOME OF HIPPOCAMPAL TRISYNAPTIC CIRCUIT AREAS: CA1, CA3 AND DENTATE GYRUS

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Hippocampus is one of the most widely studied brain structures, mainly due to its relevance to cognitive and emotional brain functions. Although some transcriptional and behavioral studies have reported differences between dorsal and ventral areas along hippocampus, the most important functional subdivision corresponds to the areas described by the hippocampal trisynaptic circuit. The trisynaptic circuit is the loop formed by the synaptic transmission through hippocampal excitatory fibers. The major input is carried by axons of the perforant path, which communicate neurons in layer II of the entorhinal cortex with dentate gyrus (DG) granule cells. Granule cells project their axons to dendrites of Cornu ammonis 3 (CA3) pyramidal cells, which, in turn, project to Cornu ammonis 1 (CA1) pyramidal cells. Finally, CA1 neurons connect again with entorhinal cortex layer V cells. CA1, CA3 and DG have shown important electrophysiological differences which seem to be associated with their different cellular characteristics and neurological functions.

Here, we provide a new methodological approach that allows the study of the synaptic function of very specific brain regions from a proteomic viewpoint. We purified postsynaptic densities (PSDs) from CA1, CA3 and DG neuropile microdissected by laser capture microdissection from frozen mouse brain histological sections. The label-free proteomic analysis of CA1, CA3 and DG PSDs revealed significant differences in the postsynaptic proteome between these three regions, supporting electrophysiological and cellular particularities reported previously. Moreover, we identified novel region-specific and region-enriched proteins involved in important signaling pathways for the synaptic function.

NOTES

O-24. CPT1C ENHANCES AMPAR SURFACE EXPRESSION BY A DEPALMITOYLATION MECHANISM

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AMPARs mediate most of fast excitatory transmission in the brain. Their dynamics – trafficking, exocytosis or endocytosis – are crucial in determining the synaptic properties of neurons and neuronal plasticity. AMPAR trafficking is mediated by specific protein-to-protein interactions. Thus, the interactions of intracellular proteins with the different core-forming subunits and/or auxiliary components of AMPARs will dictate their functional fate. We have recently shown that AMPAR GluA1 subunit interaction with CPT1C – a novel partner of AMPAR complex – is important in modulating surface expression of AMPARs and that synaptic transmission is diminished in CPT1C knockout mice. However the molecular mechanisms of such modulation remain unknown. Here we have investigated more profoundly whether CPT1C controls AMPAR trafficking. Members of the CPT1 family (CPT1A and CPT1B) catalyze the exchange of acyl groups between co-enzyme A (CoA) and carnitine to facilitate fatty acid transport through mitochondrial membranes. CPT1C is a neuronal specific form of CPT1s located in endoplasmic reticulum (ER) rather than mitochondria. A very important catalytic residue has been identified for the canonical forms of CPT1 (a Histidine that when mutated abolishes the activity of these enzymes), and this residue is conserved in CPT1C (H469). Moreover, depalmitoylating enzymes also contain a Histidine in their triad of catalytic residues. On the basis of a putative thioesterase/depalmitoylation activity of CPT1C on GluA1, we have studied the role of this histidine of CPT1C in AMPAR trafficking. Moreover we have used Palmostatin B – a compound that inhibits APT1 (a well-known palmitoyl thioesterase) – to study CPT1C role in AMPAR physiology. Our results point towards CPT1C acting as a depalmitoylating enzyme of GluA1 subunit, which accounts for the increase of GluA1 surface content at plasma membrane. Interestingly, this effect depends on CPT1C ER localization, since its miss-localization prevents that increase in AMPAR surface expression by CPT1C.

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NOTES

O-25. PYK2 IS ESSENTIAL FOR HIPPOCAMPAL SYNAPTIC PLASTICITY AND SPATIAL LEARNING AND MEMORY

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Proline-rich tyrosine kinase 2 (Pyk2) is a non-receptor calcium-dependent protein-tyrosine kinase of the focal adhesion kinase family highly expressed in the hippocampus but its role in normal and diseased brains is completely unknown. Here we characterized Pyk2 deficient mice (Pyk2-/-) and we found specific impairments in hippocampal-related learning and also in CA3-CA1 Long-term potientiation (LTP) associated with N-methyl-D-aspartate receptors subunits alterations. Accordingly, CA1 spine density was decreased and their neck length shortened. In vitro experiments by using Pyk2-/- hippocampal cells and different mutant Pyk2 constructs indicated that PSD-95 presence and stability in the excitatory synapse depends on both, Pyk2 C-terminus and the phosphorylation in its tyrosine 402. Finally, we found that Pyk2 levels and function were altered in the hippocampus of both, Huntignton's disease (HD) patients and R6/1 mouse model of the disease. Normalizing Pyk2 levels in HD mouse model rescued hippocampal memory deficits, spine pathology and PSD-95 function.

NOTES

O-26. OPTICAL CONTROL OF ENDOGENOUS RECEPTORS AND CELLULAR EXCITABILITY USING TARGETED COVALENT PHOTOSWITCHES

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Light-regulated drugs allow remotely photoswitching biological activity and enable plausible therapies based on small molecules. However, only freely diffusible photochromic ligands have been shown to work directly in endogenous receptors and methods for covalent attachment depend on genetic manipulation. Here we introduce a chemical strategy to covalently conjugate and photoswitch the activity of endogenous proteins and demonstrate its application to the kainate receptor channel GluK1. The approach is based on photoswitchable ligands containing a short-lived, highly reactive anchoring group that is targeted at the protein of interest by ligand affinity. These targeted covalent photoswitches (TCPs) constitute a new class of light-regulated drugs and act as prosthetic molecules that photocontrol the activity of GluK1-expressing neurons, and restore photoresponses in degenerated retina. The modularity of TCPs enables the application to different ligands and opens the way to new therapeutic opportunities.

NOTES

SESSIÓ 3A

MALALTIES NEURODEGENERATIVES II

ORALS

O-27. AMYLOID-BETA SOLUBLE OLIGOMERS EFFECT ON AMPA RECEPTORS: ROLE OF A-KINASE ANCHORING PROTEIN 79/150

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Amyloid-beta soluble oligomers (oAbeta) are thought to be an important source of neurotoxicity in the first stages of Alzheimer's disease (AD). However, the molecular mechanisms underlying oAbeta synaptotoxicity and memory impairment remain largely unexplained and need to be further investigated. oAbeta have been shown to disrupt synaptic structure and function, inhibiting LTP and facilitating LTD processes. Previous data from our lab showed that oAbeta disrupt AMPAR surface expression as a consequence of calcium dyshomeostasis through ionotropic glutamate receptors and the consequent GluA1 dephosphorylation by calcineurin activation.

In the present study we have analyzed how changes in synaptic activity affects A-kinase anchoring protein 79/150 (AKAP79/150) levels in cultured neurons. We observed that NMDA-mediated cLTD induces a degradation in AKAP79/150 protein levels that is dependent on proteasome activation and calcineurin (CaN)-independent. Moreover, we also saw that oAbeta are affecting AKAP79/150 levels by a mechanism related with that calcium influx. A causative relationship between the decrease in AKAP79/150 levels and the endocytosis of AMPARs is also supported. Silencing AKAP79/150 produces the expected dephosphorylation of GluA1 Ser-845 and the endocytosis of GluA1 AMPARs whereas overexpression of AKAP79/150 blocked the cLTD-mediated AMPARs endocytosis and dephosphorylation of GluA1. AKAP79/150 is a synaptic protein that has been proposed to function as a signaling scaffold associated to synaptic plasticity through the regulation of AMPAR phosphorylation, channel activity, and endosomal trafficking. Together, these results identify oAbeta-mediated changes in AKAP79/150 levels as an important factor related to the deregulation of synaptic AMPAR at early stages of AD.

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NOTES

O-28. ANALYSIS OF SYNAPTIC-RELATED MRNAS EXPRESSION IN ALZHEIMER'S DISEASE

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MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally. Recent studies have shown that deregulation of specific microRNAs could be involved in the development of Alzheimer's disease (AD). However, few studies exploring the relationship between microRNAs deregulation in AD and synaptic plasticity exist despite the involvement of some microRNAs in synaptic plasticity. Since it is believed that alterations in synaptic function are related to mild cognitive impairment, it is feasible to hypothesize that alterations in plasticity-related microRNAs could underlie AD progression. Here, levels of a small number of microRNAs involved in the regulation of AMPA receptors function were examined in mice hippocampal cultures, an AD mice model, where we reported previously changes in AMPA receptors regulation related with early deficits in learning and memory processes, and in human samples. We found increases in miR-181c-5p (40%), miR-210-3p (>60%) and miR-92a-3p (25%) expression after oAβ treatment in cultures. Furthermore, some changes in miR-181c-5p and miR-92a-3p were found in entorhinal cortex and hippocampus of APPSw,Ind six months transgenic mice. However, a compensatory mechanism (such as synaptic scaling) could occur in AD early stages since mice are still able to learn. It remains to be determined what happens later, when these mechanisms would no longer be enough. Moreover, the analysis of hippocampal human samples at different Braak stages, show an increase in miR-181c-5p and miR-92a-3p levels during AD progression. These findings indicate a possible relationship between these microRNAs and the reported changes in glutamate receptor levels and early learning and memory deficits in the AD animal model. Our results suggest that microRNAs involved in synaptic plasticity might be important factors that contribute to AD neuropathology progress.

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NOTES

O-29. RIP140: A NEW TARGET TO PREVENT NEURODEGENERATION IN X-LINKED ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is a rare disease, with fatal prognosis and no satisfactory treatment, characterized by brain inflammatory demyelination and/or axonal degeneration of corticospinal tracts in the spinal cord. It is caused by loss-of-function of the peroxisomal

transporter ABCD1. As a result, very long-chain fatty acids (VLCFA) such as C26:0 accumulate in tissues and plasma. The murine model of X-ALD (*Abcd1*$^{-}$) exhibits a late-onset axonal degeneration in spinal cord. We have previously reported that excess of C26:0 produced oxidative stress of mitochondrial origin, which compromises energy metabolism and suppresses the mitochondrial biogenesis pathway driven by the SIRT1/PGC-1α/PPARγ network, very early in the physiopathogenetic cascade.

In this study, we have identified RIP140, a novel transcriptional regulator of energy metabolism that antagonizes mitochondrial biogenesis activation by PGC-1, as being overexpressed in the X-ALD mouse spinal cord. We show that RIP140 is directly regulated by C26:0 via an oxidative stress-dependent mechanism. Further, a double knockout mouse ABCD1/RIP140 shows normalized mitochondrial respiration and bioenergetic failure, as well as an arrest of axonal degeneration and associated locomotor disabilities. Altogether, these results highlight RIP140 as a novel therapeutic target for X-ALD, and suggest that its pharmacological inhibition may be a valuable strategy to treat this and other axonopathies in which energetic homeostasis and mitochondria biogenesis may be impaired.

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NOTES

O-30. ROLE OF CRTC1 IN STRUCTURAL SYNAPTIC PLASTICITY IN THE ADULT BRAIN DURING NEURODEGENERATION

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Gene expression mediated by the cAMP-response element binding protein (CREB) regulated transcriptional coactivator-1 (CRTC1) is essential for encoding and/or storage of different forms of memory; although the underlying mechanisms are largely unknown. Recent investigations have demonstrated that a CRTC1-dependent transcriptional program related with memory and learning, synaptic plasticity, neurotransmission and neuritogenesis is deregulated in the human brain at early Alzheimer's disease (AD) stages as well as in the hippocampus of transgenic mice models of neurodegeneration. These changes correlate with hippocampal-dependent spatial memory deficits in these mice. The spatiotemporal regulation of CRTC1 in the adult brain is still unknown. In this study, we found that CRTC1 is translocated to the nucleus and activated following a characteristic spatial and temporal pattern after associative memory training. In vivo time-course evaluation of mice after contextual fear conditioning indicates that CRTC1 immediately migrates from the cytosol to the nucleus in different brain regions (CA1, CA3, basolateral amygdala and entorhinal cortex), particularly in CA3 in the hippocampus. These results suggest the existence of a dynamic topographic map of synaptic/neuronal activity during associative memory encoding that can be altered in neurological disorders. Interestingly, presenilin (PS) conditional knockout (PS cDKO) mice, a model that displays classical features of neurodegeneration occurring in AD, exhibit deficient CRTC1 nuclear translocation and transcriptional function associated with contextual memory deficits. Interestingly, CRTC1 gene therapy ameliorates transcriptional and long-term contextual memory deficits in these mice. Preliminary results indicate that PS cDKO mice exhibit dendritic spines dysmorphogenesis and that CRTC1 overexpression reverts partially these alterations. Altogether, our data suggest that CRTC1 activation may represent a novel strategy to assess simultaneously synaptic pathology and neural activity during neurodegeneration.

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NOTES

O-31. SYNTHESIS AND CHARACTERIZATION OF RHDL-RAPOJ NANOPARTICLES

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Background and hypothesis: Apolipoprotein J (ApoJ) /Clusterin is a multifunctional heterodimeric glycoprotein with chaperone functionthat interacts with Aβ oligomers, avoiding its aggregation and toxicity. These features, combined with theability of ApoJ to cross the Blood Brain Barrier (BBB), make ApoJ a good candidate to participate in the clearance of Aβ from the brain. In the present study, we propose to synthesize ApoJ-based rHDL nanoparticles (rHDL-rApoJ nanoparticles) in order to enhance the Aβ interaction ability, but also to improve

the protein stability and to provide new protective functions that have been attributed to HDLs, such us antiinflammatory properties, inhibition of apoptosis and enhancement of the endothelial cell repair. From this background, our objective is to synthesize the rHDL-rApoJ nanoparticles using recombinant human ApoJ (rApoJ) and phospholipids complexes.

Methods and Results: rHDL particles were prepared using the cholate dialysis method, based in the liposome disruption, followedby incubation with rApoJ and posterior cholate removal by dialysis. After the synthesis, rHDL-rApoJ nanoparticles were purified by ultracentrifugation in KBr density gradient. The presence of rHDL-rApoJ particles was assessed by N-PAGE and the particle size was analyzed by Dynamic Light Scattering (DLS) and Electron Transmission Microscopy (TEM), showing a diameter of 30 nm and 5 nm of height discoïdal shape. Furthermore, the conformational changes of the lipidated-protein were determined using Circular dichroism (CD). The functionality of rHDL-rApoJ particles was confirmed using Thioflavin T binding assay, preserving the property to prevent the formation of Aβ fibrils. To study their biodistribution in mice, fluorescently labeled rHDL-rApoJ nanoparticles were intravenously administrated and the localization over time was determined in vivo using an IVIS Xenogen® imager. The results confirmed their ability to accumulate in the brain.

Conclusions: We have set up a reproducible protocol to produce rHDL-rApoJ nanoparticles, which may be considered as therapeutic agents for β-amyloid-related pathologies. Furthermore, these lipid-nanoparticles are novel functional nanocarriers to deliver efficient molecules into the brain.

NOTES

O-32. DUAL THERAPEUTIC BENEFITS OF SELECTIVE-HISTONE DEACETYLASE 3 INHIBITION IN HUNTINGTON'S DISEASE MICE

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Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by motor, cognitive and psychiatric symptomatology. Cognitive deficits precede the overt motor symptoms by years in HD patients and are most highly associated with functional decline. Several studies have reported transcriptional dysregulation as an early underlying pathogenic mechanism in HD. Inhibition of histone deacetylases (HDACs) is predicted to increase histone acetylation and thereby restore normal transcription. Histone deacetylase 3 (HDAC3) has been implicated in HD mice as maintaining the aberrant transcriptional patterns that help cause some disease symptoms. Interestingly, HDAC3 negatively regulates gene transcription involved in cognitive functions such as learning and long-term memory. Furthermore, HDAC3 also helps fuel expansions of CAG repeats in human cells, suggesting that this deacetylase may power somatic CAG repeat expansions in the huntingtin gene (HTT) that are thought to drive disease progression. In view of that, we have investigated whether RGFP966, an isotype-selective inhibitor of HDAC3, improves cognitive deficits and somatic CAG expansions in the $Hdh^{Q7/Q111}$ knock-in (KI) mouse model of HD. Behavioral assessment revealed that early chronic treatment with RGFP966 completely reversed altered motor learning and impaired recognition and spatial memories in KI mice. Biochemical and genetic analysis in mouse brain samples showed that systemic administration of RGFP966 normalized the expression of memory-related genes, partially recovered striatal pathological markers and decreased aggregation of mutant huntingtin. Using molecular biology techniques to assess expansion frequencies, we could detect that chronic treatment with RGFP966 also suppressed striatal CAG repeat expansions. These novel results show that both cognitive decline and somatic repeat expansions can be modulated concurrently by pharmacological intervention and highlight HDAC3-selective inhibition as an appealing multiple-benefit therapy in HD.

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NOTES

O-33. PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE RESTORES COGNITIVE FUNCTION IN A MICE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by early cognitive impairment. Deficits in memory and learning are due to hippocampal dysfunction through alteration of synaptic plasticity. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic and multifunctional peptide that promotes synaptic plasticity, memory, hippocampal neurogenesis and neuroprotection, among others. PACAP exerts its effects via three main receptors, PAC1, VPAC1 and VPAC2. Hence, the aim of this study was to analyze PACAP as a possible therapeutic agent in order to block cognitive deficits in Huntington's disease. We first studied the protein levels of PACAP receptors in the hippocampus of the R6/1 mice model at different stages of the disease. We observed a decrease of PACAP receptors from the onset of cognitive dysfunction. The transfection of huntingtin-94-Q-GFP in neural cells (STHdhQ7/Q7 cells) also resulted in a loss of PACAP receptors meaning that their decrease seems to be associated with the expression of mutant huntingtin. Interestingly, the analysis of post-mortem hippocampal human samples showed a specific reduction of PAC1, without changes in VPAC1 and VPAC2 compared to control samples. The addition of PACAP in hippocampal cultures from R6/1 mice showed an important increase in the number of neurites and its length compared to wild-type cultures. Intranasal administration of PACAP (1 µg/µl) in WT and R6/1 mice at 15 weeks of age for 7 days blocks cognitive deficits in R6/1 mice. This effect is associated with a re-establishment of PAC1 protein levels and the expression of genes related to synaptic plasticity. These data indicate that PACAP could be a suitable candidate to promote cognitive function in HD.

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NOTES

SESSIÓ 3B: SISTEMES SENSORIAL I MOTOR I TERAPIES

ORALS

O-34. LOSS OF SYNAPSES AND CALYCEAL JUNCTIONS BETWEEN TYPE I VESTIBULAR HAIR CELLS AND AFFERENT ENDINGS ARE EARLY EVENTS DURING CHRONIC OTOTOXICITY IN RATS

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Degeneration of the sensory hair cells (HCs) is the main consequence of exposure to ototoxic chemicals. However, HC degeneration cannot explain the recovery in vestibular function reported at times during washout after chronic ototoxicity. We assessed the loss and subsequent recovery in vestibular function in rats exposed to the ototoxic compound 3,3'-iminodipropionitrile (IDPN) for four weeks, and studied in the vestibular epithelia the events associated with these functional alterations. IDPN ototoxicity resembles aminoglycoside ototoxicity, but offers a more dependable model for chronic exposure in rats. Comparison of behavioral and ultrastructural data showed that loss of vestibular function precedes loss of HCs or stereociliary coalescence. Nevertheless, a complete dismantlement of the calyceal junctions between type I HCs and calyx endings was observed by transmission electron microscopy at these early stages of functional loss in cristas and utricles. Immunofluorescence observations revealed loss of the junction proteins caspr1 and tenascin-C, and misplacement of KCNQ4. RT-PCR data indicated that the decrease in caspr1 and tenascin-C was not associated with a decrease in their mRNA expression. A good recovery of vestibular function was recorded during a 4 week washout period after exposure. This was associated with a recovery in the ultrastructural appearance of the calyceal junctions, and in the quantities and distributions of caspr1, tenascin-C and KCNQ4. We also examined the calyceal synapses by immunostaining for ribeye and either PSD-95 or GluA2 puncta. Chronic IDPN caused loss of pre- and post-synaptic puncta, and limited recovery in this effect was recorded after washout. The present data reveal new forms of damage and repair in the vestibular epithelium of adult mammals, including dismantlement of the calyceal junction and a robust capacity for its rebuilding. These findings contribute to a better understanding of the phenomena involved in progressive vestibular dysfunction and its potential recovery.

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NOTES

O-35. FOREIGN BODY RESPONSE TO INTRANEURAL IMPLANTS IN RAT

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After a limb amputation, electromechanical neuroprostheses can be used to restore the loss of function. A key component in a neuroprosthesis is the neural electrode, which interfaces specific groups of axons within the nerve to obtain different motor signals and stimulate selective populations of sensory afferents. Intraneural electrodes are able to provide a higher selectivity of recording and stimulation in comparison with extraneural electrodes, but a reduction in the functionality of electrodes with time has been reported after chronic implantation, thus limiting its prospective use. One of the factors that plays a role in this loss of function is the biological response of the nerve tissue against the electrode implant. This foreign body response (FBR) is the first reaction of the nonspecific immune system against an implanted device and is characterized by the formation of a fibrotic tissue around the electrode creating a physical separation between the interface and axons decreasing optimal electrode function.

Here, we have studied first the long-term performance of functional polyimide intraneural interfaces. While the selectivity of stimulation does not change with time, an increase of voltage is needed to elicit muscle responses during the 2 first weeks and a higher plateau is reached thereafter.

Second, we have also characterized the FBR to non-functional devices. The inflammatory response due to the implantation surgery is decreased after 2 weeks, whereas it is greater in the implanted nerves and peaks after 2 weeks. With regard to tissue deposition surrounding the implant, a tissue capsule soon appears around the devices, acquiring its maximum 2 weeks after and being remodelled subsequently. Immunohistochemical analysis reveals two different cell types implicated in the FBR in nerve: macrophages as the first cells in contact with the interface and fibroblasts that appear later on in the edge of the capsule.

Projecte EU FP7-611687 NEBIAS ("NEurocontrolled BIdirectional Artificial upper limb and hand prosthesis"), CIBERNED i TERCEL de Instituto de Salud Carlos III d'Espanya.

NOTES

O-36. US D´ONES DE XOC AL TRACTAMENT DE PUNTS GALLET MIOFASCIAL EN UN MODEL ANIMAL

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Introducció: L'alliberament d'acetilcolina (ACh) espontàniament està involucrat en la fisiopatologia de la síndrome de dolor miofascial. Sembla ser que un augment anormal en la neurotransmissió espontània pot crear un node de contracció subsinàptic al miòcit.

Objectiu: Comprovar la utilitat de les ones de xoc en el tractament dels punts gallet miofascials.

Materials i mètodes: Un estudi histològic s'ha realitzat en el múscul levator auris longus(LAL): tinció de blau de metilè i la tinció de PAS-Alcià, realitzades després d'injectar neostigmina subcutàniament i posteriors al tractament amb ones de xoc. L'estudi fisiològic s'ha realitzat amb dues tècniques: l'estudi ex vivo del múscul LAL on s'ha registrat intracel•lularment la neurotransmissió espontània (mEPPs) i la electromiografia in vivo del soroll de placa i espigues del múscul gastrocnemi prèviament tractat amb neostigmina.

Resultats: La tècnica de blau de metilè, ens ha permès descartar que les ones de xoc provoquin lesió al múscul; la tinció de PAS-Alcià ens ha permès veure com desapareixen els nodes de contracció miofascials provocats per la neostigmina. Hem vist, gràcies a les tècniques fisiològiques, que el tractament amb ones de xoc disminueix la freqüència dels mEPPs, el nombre d'àrees amb soroll de placa i el nombre d'àrees amb espigues. Tot i així cadascuna d'aquestes àrees el nombre d'esdeveniments per segon (freqüència) tant de soroll com d'espigues no varia.

Conclusió: Les ones de xoc poden ser un bon tractament de la síndrome de dolor miofascial.

Finançament. Aquest treball està finançat per una subvenció de l'Institut de Salut Carlos III, Acció Estratègica en Salut (MMS, PI13 / 02084) i el Comissionat per a Universitats i Recerca del Departament d'Innovació, Universitats i Empresa de la Generalitat de Catalunya (MB 2016 FI-B 00909).

NOTES

O-37. NEUROEHABILITATION THERAPIES IN ISCHEMIC STROKE: AN APPROACH FROM HUMAN TO MOUSE

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Neurorehabilitation therapies are the only treatments approved after acute phase of stroke to improve the functional recovery in patients. these strategies are recently known to participate in the modulation of angiogenesis and tissue remodeling. in fact, metalloproteinases (mmps) are crucial in brain repair and preliminary results from our lab show that mmps could act as prognosis markers of motor improvement during intensive rehabilitation therapies in stroke patients. in this context, our aim is to establish a post-stroke neurorehabilitation model (rm) in mice and study neurorepair at molecular and cellular levels.

after inducing cerebral ischemia by middle cerebral artery occlusion (mcao) in mice neurorehabilitation groups were: non-rehabilitation (n=6), pasta matrix (focused motor task) (n=8), treadmill (forced motor task) (n=6) and combination (pasta matrix+treadmill) (n=7). after randomization, mice received treatment at 48 and 72 hours and euthanized at day 4. body weight and forelimb strength were measured at different time points whereas infarct size was measured at the end of the study. additionally, brain mmps (mmp2, mmp3, mmp8, mmp9 and mmp12) were analyzed by a milliplex® kit and endothelial progenitor cells (epcs) primary cultures from spleen were performed. epcs were counted as spindle-like cells together with total cells at day 5 of culture.

forelimb force decreased at day 4 only in non-rehabilitation and combination treatment groups ($p<0,05$) being infarct volume similar between groups and epcs counts did not change between groups in our rm at day 4. interestingly, brain protein levels of mmp2, mmp3, mmp8 and mmp9 were higher in the infarcted vs. non-infarcted cortex ($p<0,05$) , but no differences were found among rehabilitation groups.

the proposed forced and focused motor tasks might be good rms post-ischemia although the underlying mechanisms are still unknown. future long-term investigations are required to determine angiogenesis modulation during neurorehabilitation therapies.

NOTES

O-38. NEUREGULIN 1-ERBB MODULE IN C-BOUTON SYNAPSES ON SOMATIC MOTOR NEURONS: MOLECULAR COMPARTMENTATION, CHANGES DURING ALS AND RESPONSE TO PERIPHERAL NERVE INJURY

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Lower motoneurons (MNs) are the neuronal population involved in the control of voluntary motor activity. MN degeneration is the disease hallmark in Amyotrophic Lateral Sclerosis (ALS), where a progressive disconnection of MNs from their muscle targets is produced leading a muscular paralysis. There is no effective therapy for ALS and since the diagnosis, the patients have a low life expectancy.

Electric activity of motor neurons (MNs) appears to play a role in determining cell vulnerability in MN diseases. MN excitability is modulated by cholinergic inputs through C-type synaptic boutons. C-boutons contact lower MNs and display an endoplasmic reticulum-related subsurface cistern (SSC) adjacent to the postsynaptic membrane.

Besides cholinergic molecules, a constellation of proteins involved in different signal- transduction pathways are clustered at C-type synaptic sites (M2 muscarinic receptors, Kv2.1 potassium channels, Ca2+ activated K+ [SK] channels, and sigma-1 receptors [S1R]), but their collective functional significance is unknown. We have previously suggested that neuregulin-1 (NRG1)/ErbBs-based retrograde signalling occurs at this synapse. Furthermore, mutations in ErbB4 have been associated with ALS, suggesting that this pathway may have an important role in the disease. To better understand the signalling through C-boutons, we performed an analysis of the distribution of C-bouton-associated signalling proteins. We show that, within SSC, S1R, Kv2.1 and NRG1 are clustered in highly specific, non-overlapping, microdomains, whereas ErbB2 and ErbB4 are present in the adjacent presynaptic compartment. This organization may define highly ordered and spatially restricted sites for distinct signal-transduction pathways. SSC associated proteins are disrupted in axotomized MNs together with the activation of microglia, which displayed a positive chemotactism to C-bouton sites. Preliminary data indicate that this can also occur in ALS in a similar way. This indicates that C-bouton associated molecules are also involved in neuroinflammatory signalling in diseased MNs, emerging as new potential therapeutic targets.

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NOTES

O-39. PAIN RESPONSES AND BEHAVIORAL DISTURBANCES IN CD1 MICE DURING ACUTE AND CHRONIC PHASES AFTER SPINAL CORD INJURY

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Patients suffering from neuropathic pain secondary to spinal cord injury (SCI) experience not only pain sensation but also some affective disorders. Therefore, research in new animal models has emerged as a need to assay promising therapeutic strategies to treat both neuropathic pain and potential behavioral disturbances associated to SCI. The present study aimed to characterize these disturbances during the acute and the chronic phase of an SCI mice model. Mechanical allodynia (von Frey test) and thermal hyperalgesia (Hargreaves plantar test) were evaluated at 7,14,21,28 dpi (acute phase) and at 28, 60, 90 dpi (chronic phase). Emotional alterations such as depression-like behavior, social interaction and anhedonic-like behavior were assessed at 28 dpi and at 90 dpi.

In the acute phase of the disease, mechanical allodynia and thermal was evidenced at least until 28 days post injury (dpi) and at that time no significant differences in anxiety-like behaviour were found whereas a mild social interaction alteration was observed. This results suggest that the hypersensitivity found at 28 dpi can induce alterations in the mood of the animal resulting in a depression-like behaviour manifestation. In the chronic period of the lesion, mechanical allodynia was not detected at any time point, while thermal hyperalgesia lasted until 60 dpi and not observed at 90 dpi. Additionally, tendencies toward significance were found in both social and anhedonic-like behaviors 90 dpi despite the absence of pain responses. This data may suggest that chronic central neuropathic pain may cause emotional disturbances that could be persistent over time despite pain responses subside. The present animal model may be suitable for further pharmacological studies to relieve the main pain responses and their behavioural alterations associated to SCI, which is a major source of suffering and requires special clinical care.

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NOTES

SESSIÓ 4A

RECEPTORS I SENYALITZACIÓ CÉL.LULAR

ORALS

O-40. GLUA1 TRAFFICKING IS REGULATED BY MALONYL COA

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Carnitine Palmitoyltransferase 1C (CPT1C) is an isoform exclusively found in the brain that, unlike the other two mitochondrial isoforms, is localized in the endoplasmic reticulum and has a residual catalytic activity. Although CPT1C is not involved in mitochondrial transport of fatty acids for their oxidation, this isoform is also able to bind malonyl-CoA, an intermediary metabolite of fatty acid synthesis that decreases during fasting conditions. It has been suggested that CPT1C might be a sensor of malonyl-CoA levels in the brain.

Despite the biochemical function of CPT1C remains unknown; recent studies have demonstrated that this protein is one of the constituents of AMPA type glutamate receptors (AMPARs) involved in learning processes. Here, we determined whether basal GluA1 trafficking changed during metabolic stressed conditions in cortical neurons, and whether CPT1C was involved in this regulation. Neither total GluA1 levels nor the strength of CPT1C-GluA1 interaction were altered after 2 hours of TOFA treatment (an inhibitor of malonyl-CoA synthesis). However, biotinylation assays indicated that surface GluA1 levels decreased when neurons were treated with TOFA for 2 hours. By contrast, CPT1C KO cortical neurons did not respond properly to changes in malonyl-CoA levels. These results suggest an interesting role of CPT1C as a sensor of malonyl-CoA and a regulator of GluA1 trafficking during metabolic stressed conditions.

NOTES

O-41. D_2 RECEPTOR CROSSTALK WITH METABOTROPIC GLUTAMATE RECEPTORS REGULATES DOPAMINE SYNTHESIS IN RAT BRAIN

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Dopamine receptors play a key role in motor activity and goal-directed behaviors and are relevant for the treatment of diverse disorders, including schizophrenia and Parkinson's disease. G protein-coupled receptors are known to form homo- and heterodimers at the plasma membrane, but the function of such receptor oligomers is relatively unknown. Here we hypothesized that heteromerization between D_2 receptors and other receptors would alter responses to D_2 agonists, which we measured with a sensitive radioisotopic methodology assessing D_2 receptor inhibition of rat brain dopamine synthesis. The inhibitory effect of D_2 receptor full agonist quinpirole could not be modified with $mGlu_5$ negative allosteric modulators MTEP or fenobam or with the $GABA_B$ antagonist CGP55845. In contrast, MTEP completely blocked the effect elicited by the D_2 partial agonists (-) PPP and aripiprazole. In addition fenobam and MTEP, but not the $GABA_B$ antagonist CGP55845, blocked the effect elicited by (-) PPP. The simplest explanation for this crosstalk between D_2 and $mGlu_5$ receptors in the regulation of dopamine synthesis is a membrane interaction or heteromerization that has already been described in cultured cells[1]. Such membrane interaction appears to affect only responses to D_2 partial agonists, but not full agonists. These results could be relevant for the treatment of neuropsychiatric disorders, where D_2 receptors are important targets of pharmacotherapy.

[1] Cabello N, Gandía J, Bertarelli DC, Watanabe M, Lluís C, Franco R, Ferré S, Luján R, Ciruela F. Metabotropic glutamate type 5, dopamine D2 and adenosine A2a receptors form higher-order oligomers in living cells. J Neurochem. 2009 Jun;109(5):1497-507. doi: 10.1111/j.1471-4159.2009.06078.x.

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NOTES

O-42. COCAINE MODULATES OREXIN-CRF RECEPTOR HETEROMERS SIGNALLING IN THE VENTRAL TEGMENTAL AREA

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Release of the neuropeptides corticotropin-releasing factor (CRF) and orexin-A in the ventral tegmental area (VTA) play an important role in stress-induced cocaine-seeking behavior. We provide evidence for pharmacologically significant interactions between CRF and orexin-A that depend on oligomerization of CRF1 and orexin OX1 receptors. CRF1R-OX1R heteromers are the conduits of a negative crosstalk between orexin-A and CRF as demonstrated in transfected cells and in the VTA, where they significantly modulate dendritic dopamine release. The cocaine target sigma σ1 receptor (σ1R) also associates with the CRF1R-OX1R heteromer. Cocaine binding to the σ1R-CRF1R-OX1R complex promotes a long-term disruption of the orexin-A-CRF negative crosstalk. Through this mechanism cocaine sensitizes VTA cells to the excitatory effects of both CRF and orexin-A, thus providing a mechanism by which stress induces cocaine seeking.

NOTES

O-43. LOCAL PHOTOACTIVATION OF A MGLU5 RECEPTOR ALLOSTERIC MODULATOR REDUCES PAIN IN MICE

López-Cano M[3,4*], Font J[1,2*], Notartomaso S[5], Scarselli P[5], Bresolí-Obach R[6], Battaglia G[4], Malhaire F[7,8], Rovira X[7,8], Catena J[1], Giraldo J[2], Pin JP[7,8], Fernández-Dueñas V[3,4], Goudet C[7,8], Nonell S[6], Nicoletti F[5,9], Llebaria A[1,10], Ciruela F[3,4,10]

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[10]These authors jointly directed this work.
**These authors contributed equally.*

Pain is an unpleasant sensory and emotional experience associated with actual of potential tissue damage. Indeed, pain can be considered a disease if it becomes chronic and thus new pharmacotherapeutic strategies are needed for such condition. Interestingly, accumulated evidences suggested that the mGlu5 receptors play a key role in transmission through the pain neuraxis, thus being good candidates for pharmacological interventions. Thereby, selective NAMs of mGlu5 receptors have consistently shown analgesic activity in experimental animal models of inflammatory pain. Nevertheless, those receptors are widely expressed at different levels of the pain neuraxis and they could have different effect in pain sensation, since they may be pro- or antinoceptive, depending on its anatomical location, thus a spatial mode of action is needed. An alternative and novel approach would consist in systemically inject inactive photoactivable mGlu5 NAM-based drugs (caged-compounds) whose activation may rely on light irradiation (photo-uncaging) in a discrete brain regions, allowing the release of the active compound in a time and space controlled manner. Using that approach, we synthesized a caged derivative (JF-NP-26 compound) using the chemical structure of raseglurant (mGlu5 receptor NAM) as a moiety. JF-NP-26 compound can be photo-activated by 405nm light irradiation releasing the raseglurant compound. Here, we evaluated the ability of the JF-NP-26 compound to preclude in a light-dependent manner the mGlu5 activity in cultured cells and neurons. Importantly, JF-NP-26 showed antinociceptive activity in an animal model of pain upon irradiation both at the peripheral (i.e. hind paw) and central level (i.e. thalamus). Overall, these results demonstrated for the first time the usefulness of using light in combination with photochromic drugs for treatment of pain.

NOTES

O-44. STRIATAL PHOTOACTIVATION OF AN ADENOSINE A_{2A} RECEPTOR ANTAGONIST CAGED CONTROLS LOCOMOTION ACTIVITY IN MICE

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The adenosinergic system operates through G protein-coupled adenosine receptors, which have become promising therapeutic targets for a wide range of pathological conditions, such as Parkinson disease. However, the ubiquity of adenosine receptors and the eventual lack of selectivity of adenosine-based drugs have frequently diminished their therapeutic potential. Optopharmacology can help to sort out this issue. Accordingly, here we aimed to develop the first generation of novel light-sensitive caged adenosine receptor ligands, allowing the spatiotemporal control of receptor functioning (i.e., receptor activation dependent on location and timing). Therefore, we synthesized the MRS7145, a SCH442416 derivative coumarin-blocked on the N^6 substituent. In dark conditions no activity is observed while upon photo-stimulation with violet light (405 nm), the SCH442416 is effectively released and can freely behave as a potent selective adenosine A_{2A} receptor ($A_{2A}R$) antagonist. Interestingly, the properties of this compound have been demonstrated on this study not only in living cells but also on a brain fiber-optic implanted mouse-model. Thus, MRS7145 enables to block the agonist-induced generation of cAMP after being photo-released. Furthermore, MRS7145 induces hyperlocomotion after direct optical stimulation of the dorsal striatum of a mice brain. Overall, the development of light-operated adenosine receptor ligands expands the pharmacological toolbox in support of research and possibly opens new pharmacotherapeutic opportunities.

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NOTES

O-45. NEUROMUSCULAR ACTIVITY MODULATES THE SIGNALING OF THE M_2 SUBTYPE MUSCARINIC CHOLINERGIC RECEPTOR ON PKC AND ON SNAP25 AND MUNC18-1 PHOSPHORYLATION

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In the last few years, there has been increasing evidence that muscarinic receptors (mAChR) play an important role in regulating the release of acetylcholine in various neural tissues (Minic et al., 2002). However, how an increase of nerve-induced muscle contraction modulates neuromuscular synaptic function through acetylcholine and its muscarinic autoreceptors remains poorly understood. Recently, it has been identified that when a continuous electrical stimulus is imposed to neuromuscular junction, protein kinase C (PKC) family modulates neurotransmitter release. Also, the resulting muscle contraction has an important impact on the levels of presynaptic PKC isoforms such as cPKCβI and nPKCε (Besalduch et al., 2010; Obis et al., 2015a). Accordingly, the present study hypothesizes that the M2 subtype mAChR signaling constitutes a mechanism through which muscle contraction and synaptic stimulation regulate neurotransmission, involving an interplay with the M1 subtype, several PKC isoforms and proteins of the synaptic vesicle fusion pathway (Munc18-1 and SNAP25). We used immunohistochemistry and confocal microscopy to demonstrate that both M1 and M2 mAChR are located in the nerve terminals at the NMJ and in the teloglial cell. Also, we found that both pre- (only nerve terminal activity) and postsynaptic (muscle contraction) neuromuscular activities play a crucial role in modulating muscarinic signaling. In the first place, these activities modulate differently M1 and M2 mAChR protein levels. Furthermore, our results show that M2 mAChR inhibition of PKC isoform is dependent of the ongoing neuromuscular activity, being nPKCε inhibited by M2 mAChR under basal conditions and with the presence of nerve-induced contraction and cPKCβI inhibited under nerve-induced stimulus without contraction. Additionally, M2 mAChR inhibition also resulted in a decrease in SNAP25 and Munc18-1 phosphorylation. Together, these results provide a mechanistic insight into how M2 mAChR could regulate exocytotic machinery and how synaptic activity-induced muscle contraction can modulate its effects.

NOTES

O-46. QUANTITATIVE AND TIME-DEPENDENT REGULATION OF THE ALTERNATIVE SPLICING OF EXON 23A OF THE NEUROFIBROMATOSIS TYPE 1 GENE IS ESSENTIAL FOR A CORRECT NEURONAL DIFFERENTIATION PROCESS

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The role of neurofibromin, the product of the Neurofibromatosis Type 1 (NF1) gene, in cell physiology is still not completely understood. The Ras-GAP activity of neurofibromin is its best characterized biochemical function, which is regulated by the alternative splicing of exon 23a (E23a) that can modulate a 10-fold difference of activity from E23a inclusion (less GAP activity) to E23a exclusion (more GAP activity). In this work we intended to better understand the role of alternative splicing of E23a during neuronal differentiation, by using antisense oligonucleotides, with the aim that in the future it could provide information on the learning and cognitive issues related to this disease. We developed a Phosphorodiamidate Morpholino Oligomers (PMOs)-based system that successfully allowed to force the expression of type II (+E23a) or type I (-E23a) NF1 isoforms without altering the physiological expression levels of NF1 mRNA in PC12 cells in the presence or absence of Nerve Growth Factor (NGF). Our results demonstrate that any alteration of the NGF-induced ratio between type I/II isoforms, either in a quantitative or time-dependent manner, interfered with the correct neuronal differentiation process, in particular, altering the correct formation of neurites, as well as the proper regulation of the RAS/MAPK and cAMP/PKA signaling pathways. This is the first time that the GAP activity of neurofibromin is shown to be involved in the regulation (directly or indirectly) of the cAMP/PKA pathway. The alteration of the natural E23a alternative splicing also impeded the proper neuronal differentiation process in other neuronal model systems, such as the embryonic H19-7 hippocampus cells.

All together, the results indicate that the regulation of the alternative splicing of exon 23a of the NF1 gene allows the fine-tuning of the RAS/MAPK and cAMP/PKA pathways through its GAP activity in a coordinate and opposite way along the time-dependent process of neuronal differentiation.

NOTES

SESSIÓ 4B

COGNICIÓ, COMPORTAMENT I TRASTORNS MENTALS

ORALS

O-47. CDK5-DA SIGNALING HAS A KEY ROLE IN DEPRESSIVE LIKE BEHAVIOURS MANIFESTED AT EARLY DISEASE STAGE IN HD

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Depression is one of the most common psychiatric symptoms in Huntington´s disease (HD), estimated to occur in between 20 and 50 % of HD carriers. Notably, mood disturbances may precede the onset of cognitive changes and motor disturbances by several years making depression one of the earliest symptoms in HD. Several evidences indicate that depressive symptoms appear in the early stages of the disease and can prevail throughout the clinical course with devastating consequences as they may exacerbate cognitive and motor symptoms. Studies about the biochemical, molecular and cellular bases involved in the pathophysiology of depression in HD are scarce. Several evidences have shown that both anxiety and depression could be regulated by the activity of Cdk5, a multifunctional kinase involved in a wide range of neuronal functions from synaptic plasticity to cell survival. Increases in the activity of this kinase has been associated with altered neuronal processes, cell death and dysfunction characteristic of different neuropsychiatric and neurodegenerative diseases. Therefore we hypothesize that aberrant activation of Cdk5 could be involved in the depressive symptoms charateristics of the early stages of HD. In this study we demonstrated that our HD KI mice dysplayed depressive-like behaviors without an anxiety component starting at 2 months of age and preceeding cognitive deficits (4-6 months of age and motor impairments (8 months of age) by several months. This early depressive behavior was associated with alterations in the dopaminergic and seratoninergic metabolism. Importantly, in this study we used genetic and pharmacological approaches to define whether Cdk5 is a good drug target for early treatment of depressive symptoms in HD. Our results demonstrate that cdk5 play a critical rol in the development of depressive like-behaviors by modulation of dopamine signaling.

NOTES

O-48. PPM1F IS REGULATED BY STRESS AND ASSOCIATED WITH ANXIETY AND DEPRESSION

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Using convergent genomic approaches, we aimed to identify genes underlying psychological sequelae of exposure to stressful or traumatic life experiences. Initially, we identified six genes significantly differentially expressed in the amygdala of stressed mice vs. controls a week after immobilization stress. We then examined expression of these same genes in peripheral blood of humans with comorbid PTSD and depression (PTSD&Dep), finding PPM1F significantly downregulated in cases vs. controls after adjusting for gender, age, and population substructure. Consistently, in an independent cohort, PPM1F expression also inversely correlated with anxiety symptoms. To follow up, we examined associations between the single nucleotide polymorphisms (SNPs) of PPM1F and PTSD&Dep, finding that rs17759843 significantly associated with PTSD&Dep after adjusting for sex and population substructure. We further demonstrated that this SNP influences PPM1F expression in both human brain and blood, serving as an expression quantitative trait locus. Given the reported mechanistic link between PPM1F and CAMKII, we then examined CAMKII expression in human blood. We found that CAMK2G had significantly lower expression in PTSD&Dep cases vs. controls after adjusting for gender, age, and population substructure. Camk2g was also differentially regulated in the amygdala of stressed mice. Taken together, our data suggest that a pathway involving PPM1F and CAMKII is involved in the mechanism of stress and trauma-related anxiety and depressive-like symptoms across species.

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NOTES

O-49. CONTRIBUTION OF THE 14-3-3 GENE FAMILY TO AUTISM SPECTRUM DISORDER

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Objectives: We recently identified a heterozygous 1-bp insertion in the *YWHAZ* gene (c.659_660insT, p.L220F*fs**18) in two brothers with Autism Spectrum Disorder (ASD) (Toma *et al*. Mol Psychiatry 2014;19:784). This gene encodes 14-3-3ζ, one of the seven isoforms of the 14-3-3 protein family. In this study we aimed to *(1)* functionally characterize the disruptive mutation identified in *YWHAZ*; and *(2)* evaluate the possible implication in ASD of all members of this gene family (*SFN*, *YWHAQ*, *YWHAG*, *YWHAZ*, *YWHAB*, *YWHAH*, *YWHAE*). Methods: Normal and truncated 14-3-3ζ were expressed as fusion proteins in *E. coli*, where their solubility was tested. Furthermore, Biacore experiments were performed to assess the affinity of the truncated 14-3-3ζ with the well-known interactor Ser19-phosphorylated tyrosine hydroxylase (TH-Ser19P). To test the possible involvement of common and rare variants of the 14-3-3 genes in ASD we performed, respectively, *(a)* a case-control association study in 727 ASD patients and 714 controls, by using tagSNPs covering the seven genes; and *(b)* resequencing of all these genes in 285 ASD patients. Results: Truncated 14-3-3ζ presented a decreased solubility and lost its affinity with TH-Ser19P. No common variants were found associated in our study. The mutation screening identified two potentially damaging variants in *SFN*. Conclusions: These results suggest a possible dominant negative effect of the truncated 14-3-3ζ. The genetic study of the 14-3-3 gene family did not identify other risk variants in the *YWHAZ* gene. Although potential mutations were identified in *SFN*, further experiments are needed to test its possible contribution to ASD.

NOTES

O-50. A *DE NOVO GRIN2B* MISSENSE MUTATION CAUSING RETT-LIKE SEVERE ENCEPHALOPATHY IS ATTENUATED BY D-SERINE DIETARY SUPPLEMENT

Soto D[1,2,3], Olivella M[4], Alcón C[2,5], Grau C[6], Gómez de Salazar M[6], Gratacòs-Batlle E[1,2,3], Ramos-Vicente D[7,8], Ciruela F[6], Bayés À[7,8], Sindreu C[2,5], López-Sala A[9], Armstrong J[10], García-Cazorla À[9,10], Altafaj X[6]

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N-Methyl D-Aspartate subfamily of glutamate ionotropic receptors (NMDARs) are activated during fast excitatory transmission and they have been proved to be key elements in synaptic plasticity, synaptogenesis and neuron survival. Several genetic studies have identified 'de novo' NMDAR mutations in patients with neurodevelopmental diseases (including severe encephalopathies, autism, intellectual disability) as well as psychiatric disorders. In this work we report a case study of a 4 years-old Rett-like patient with a severe encephalopathy. The genetic studies of this patient (WES and Sanger sequencing) showed the presence of a missense de novo mutation of GRIN2B(p.P553T) coding for the GluN2B subunit of NMDARs. Given the key role of GluN2B subunit in the very early stages of synaptogenesis, we hypothesized that this mutation could be leading to neuronal dysfunction and, subsequently, its normalization would potentially ameliorate the patient's symptomatology. In heterologous expression systems, GluN2B(P553T) mutant construct do neither affected NMDAR oligomerization nor their surface expression in primary neuronal cultures. However, electrophysiological studies showed that although functional, the mutant receptor displayed a significantly reduced channel conductance concomitant with a strong reduction of NMDA-evoked current density. These data are in agreement with our structural molecular model, and strongly suggest the hypo-functionality of mutant NMDARs that, potentially, could be rescued throughout the enhancement of their activity. In accordance with this hypothesis, in vitro administration of D-serine, a physiological NMDAR co-agonist, displayed a significant increase of NMDA-evoked currents of mutant receptors. Next, a clinical trial with dietary supplement of D-serine was performed. Importantly, after nine-months dietary supplement of D-serine, the patient showed an increase of serine plasma levels, together with a noteworthy clinical improvement. Our results show the possibility to enhance the hypo-functionality of glutamatergic transmission as a therapeutic approach to attenuate cognitive and motor impairment in early childhood.

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NOTES

O-51. PHOSPHATASE DUSP6 REGULATES HIPPOCAMPAL ERK1/2 ACTIVATION AND LONG-TERM MEMORY

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Transient activation of the Erk/MAP kinase pathway in hippocampal CA1 neurons is essential for contextual memory. However, little is known about the phosphatase(s) that directly regulate Erk1/2 during memory formation. Here we show that Dusp6 (a dual-specificity Y/T-phosphatase) is selectively expressed in postsynaptic compartments of CA1 and CA3 pyramidal cells, including dendritic spines. Dusp6-KO mice showed marked deficits in contextual fear conditioning, object recognition and spatial reference memory. Notably, local knockdown of Dusp6 in area CA1 reproduced the contextual deficits observed in global KO mice. Binding between Dusp6 and Erk1/2 transiently increased after contextual training, suggesting that Dusp6 regulates the time course of Erk1/2 activation. In keeping with this, Dusp6-KO mice showed increased levels of phospho-Erk1/2 in CA1 cells, and impaired induction of downstream phospho-CREB by training. Dusp6-KO mice also showed elevated levels of PSD-95, GluN2A and pY-GluN2B, indicating possible effectors underlying the memory deficits. Collectively, the results indicate that Dusp6 plays a critical role in hippocampus-dependent long-term memory, and underscore the importance of Dusp6 in Erk1/2 regulation in neurons.

NOTES

O-52. ABSOLUTE QUANTIFICATION OF SYNGAP C-TERMINUS VARIANTS IN MOUSE CORTEX DURING POSTNATAL DEVELOPMENT BY TARGETED PROTEOMICS

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Mutations in SYNGAP1 gene cause a form of non-syndromic intellectual disability yet, its role in brain development is poorly understood. This gene has a rather complex transcriptional regulation giving rise to several isoforms that vary in their N- and C-terminus (C-term). Although different isoforms are supposed to have different roles nothing is known about their different properties or expression patterns. Here we elucidate for the first time the expression pattern of all four SynGAP C-term variants (Alpha1, Alpha2, Gamma and Beta) throughout mice cortical development by establishing a specific SynGAP immunoprecipitation protocol and using Single Reaction Monitoring Mass Spectrometry (SRM-MS). Our data indicates that, overall, SynGAP protein expression in cortex keeps increasing during development up to postnatal day 21 (P21), remaining then constant until adulthood. Nevertheless, when looking into C-term variants expression individually we observe certain differences among them. Based on their abundance, different variants could have prominent roles during specific development stages. Strikingly, the most studied variants (alpha1 and alpha2) are the least abundant, especially early in development as compared with Beta and Gamma ones. Particularly noticeable are the low levels of alpha2 found in murine cortex. These results emphasise the need to better understand Beta and Gamma variants function in development, as these could have important implications for intellectual disability.

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NOTES

O-53. A STUDY OF THE EFFECT OF THE DISC1 GENE IN THE VULNERABILITY FOR SCHIZOPHRENIA-SPECTRUM DISORDERS THROUGH ITS ASSOCIATION WITH NEURODEVELOPMENT MARKERS

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Background: Disrupted-in-Schizophrenia 1 (DISC1) is a candidate gene for schizophrenia-spectrum disorders (SSD) with a range of functions relating to neurodevelopment (Ayalew et al., 2012). This study aimed to examine i) the relation between the DISC1 and the risk for SSD and ii) whether variations in this gene are associated with the presence of neurodevelopment markers such as dermatoglyphic anomalies (Golembo-Smith et al., 2012).

Methods: The sample consisted of 383 healthy controls and 693 SSD patients. Six SNPs (TRAX and DISC1 genes) were genotyped. Bilateral fingers and hand prints were obtained in a subset of the sample. Palmar a–b ridge count (ABRC) and its fluctuating asymmetry (FAABRC) were examined. Haploview v4.1 was used to estimate the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium. Case-control and dermatoglyphic association analyses were performed using PLINK. DISC1 relationship with dermatoglyphic markers was tested by means of linear regression models (adjusted for sex), separately in patients and controls.

Results: All SNPs were in HWE and similarly to previous studies three haplotypes were described. Haplotypes were not associated with the risk for SSD. The haplotype AG (rs751229-rs3738401) was positively associated with the FAABRC in controls (p=0.018); while no significant results were found in patients.

Conclusions: The AG haplotype (rs751229-rs3738401) within the DISC1 has been associated with higher scores in FAABRC in healthy controls, but not in patients. Since these dermatoglyphic anomalies are associated with development disturbances, these results suggest that DISC1 might modulate the neurodevelopment in a differential effect depending on the genetic and environmental background of the individual. Remarkably, this haplotype has been previously associated with brain development related markers such as reductions in the prefrontal cortex grey matter density (Cannon et al. 2005).

NOTES

PÒSTERS SESSIÓ 1

CEL·LULES GLIALS I NEUROINFLAMACIÓ

PÒSTERS

P-1. CAMP EFFECTS ON POLARIZATION OF MICROGLIA AND MACROPHAGES TO PRO-INFLAMMATORY OR ANTI-INFLAMMATORY PHENOTYPES

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Microglia (µglia) and infiltrating macrophages (MØ) are implicated in the inflammatory response to neurodegenerative diseases by secretion of toxic molecules and antigen presentation to cytotoxic lymphocytes. They display regenerative functions by debris clearance by phagocytosis and growth factors secretion. There appears to be equilibrium in the lesion between cells that either exacerbate tissue injury or promote CNS repair. In vitro studies show that this equilibrium can be shifted by chemical stimuli. “Classically activated” M1 µglia/MØ or “M1 spectrum” are considered pro-inflammatory and can be induced by stimuli such as LPS and the pro-inflammatory cytokines IFNγ or TNFα. The “alternative activation” to the M2 phenotype or to the “M2 spectrum” has anti-inflammatory and regenerative properties through secretion of anti-inflammatory cytokines and trophic factors and debris clearance. We have studied the influence of cAMP levels on the conversion of microglia cell line Bv-2 or macrophage cell line RAW264.7 to M1 or M2 phenotype when cells are grown in presence of pro and anti-inflammatory stimuli. The elevation of cAMP levels has been obtained by the use of cAMP-phosphodiesterase 4 (PDE4) inhibitors rolipram, roflumilast or apremilast or by synthetic cAMP analogs , dibutyryl cyclic AMP or 8-bromo-2’O methyl-cyclic AMP. Cells were preincubated with PDE4 inhibitors , then stimulated under M1 conditions (10ng/mL LPS, 20ng/mL IFN-γ) or under M2 conditions (20ng/mL IL-4 for microglia or 20ng/mL each IL-4 and IL-10 for MØ). We analyzed the results by immunocytochemistry with phenotype specific antibodies (iNOS for M1 cells and YM-1 or mannose receptor for M2 cells) and also by determination of the corresponding enzymatic activities (NO production by M1 phenotype or arginase I activity by M2 phenotype). We will present data on the influence of cAMP levels on the M1/M2 cell polarization when cells are grown in the presence of pro- or anti-inflammatory stimuli.

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P-2. FULL MICROGLIAL ACTIVATION AFTER IFN-GAMMA/LPS-MEDIATED PRIMING LEADS TO INCREASED PHAGOCYTOSIS OF DOPAMINERGIC CELLS IN AN IN VITRO MODEL OF PARKINSON'S DISEASE

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Microglial cells constitute the first barrier of the innate immune response in the brain and act by continuously scanning the surrounding extracellular space and communicating with other cells, including neurons. Changes in microglial phenotype and function are observed during almost all neuropathological conditions like degenerative diseases such as Parkinson's disease (PD).Our aim is to determine whether microglial cells when fully activated after priming, are able to increase the phagocytosis of dopaminergic neurons in an in vitro model of Parkinson's disease (PD).

We studied the activation that leads to phenotypical changes of BV-2 microglial cell line. Firstly, we analyzed the response of these cells to pro-inflammatory agents such as the antigenic molecule lipopolysaccharide (LPS) or IFN-gamma, simulating the neuro-inflammatory environment that occurs during PD. The release of nitrites by these cells was studied as an indicator of their activation and immunocytofluorescence essays allowed us to see morphological changes due the aforementioned activation. Previously activated microglial cells were set up in a co-culture with PC12 cells (dopaminergic neuron-like cells) as an *in vitro* model of the disease, allowing us to see whether BV-2 cells increase their phagocytic domains over PC12 cells. Results indicate that treatment with IFN-gamma alone provokes a release of low concentrations of nitrites. However, full activation of microglial cells was achieved when priming cells with both IFN-gamma and LPS, as seen by the elevated nitrite release. Importantly, microglial cells were able to spontaneously phagocytose PC12 cells over the course of 24 hours and interestingly, engulfments were significantly elevated when BV-2 cells were fully activated after priming.

These results suggest that microglial priming is an important factor in the inflammatory-mediated dopaminergic neurodegeneration and will help us to understand the role that the immune response plays in parkinsonism, highlighting the significance of the inflammatory component in PD therapy.

This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF2013-45178-P and SAF2015-64123-P).

P-3. CCL23: A NEW CHEMOKINE PRESENT IN BRAIN AFTER CEREBRAL ISCHEMIA MIGHT PLAY A ROLE AS A BLOOD BIOMARKER FOR BRAIN DAMAGE AND STROKE OUTCOME

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CCL23 plays a fundamental role in the modulation of the immune response. Circulating CCL23 levels have been found elevated in several inflammatory diseases, but nothing is known about the role of this chemokine in the stroke-associated inflammatory process. Our aim was to evaluate the potential of this chemokine for stroke diagnosis and prognosis and complement it with a molecular study in experimental stroke models.

CCL23 blood levels were evaluated in a total of 369 individuals, including ischemic stroke and stroke-mimicking patients within <4.5h from symptoms onset. Both disease groups presented higher CCL23 levels than healthy volunteers ($p<0.001$ for both comparisons). Although strokes could not be differentiated from mimics ($p=0.173$), CCL23 levels identified any type of cerebral injury ($p=0.006$) and showed an increase in blood levels at 24h after stroke ($p<0.001$). Moreover, in stroke patients, baseline level of circulating CCL23 resulted an independent predictor of mortality after 3 months of stroke onset (ORadj: 26.969 [3.251-223.729], $p=0.002$) and disability at hospital discharge (ORadj: 29.073 [1.657-510.02], $p=0.021$).

In vivo, CCL23 levels were increased 2h after stroke in cerebrospinal fluid, but not in plasma, from rats submitted to cerebral ischemia ($p=0.006$ and $p=0.851$, respectively, compared to sham-operated animals). The expression state of the CCL23 rodents' counterparts, CCL6 and CCL9, resulted to be up-regulated in brains from ischemic rats 2h after stroke ($p=0.095$ and $p=0.063$, respectively) and remained increased in mouse brains 24h after the challenge ($p=0.068$ and $p=0.038$, respectively), corroborating the brain provenance of this chemokine at early time points after cerebral ischemia. Conversely, 5 days after stroke, the presence of CCL23 in human brains was only seen on granulocytes infiltrated into the brain parenchyma by that time.

Thus, CCL23 might have a dual role in the inflammatory processes associated to cerebral injury and its baseline levels might be good predictors of stroke outcome.

P-4. CYCLIC CGMP PROMOTES REMYELINATION IN LYSOPHOSPHATIDYLCHOLINE DEMYELINATED ORGANOTYPIC CEREBELLAR CULTURES

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Using experimental autoimmune encephalomyelitis (EAE) induced by MOG immunization in mice as a multiple sclerosis model we have previously shown that daily treatment with the cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor sildenaï¬ l at peak disease rapidly ameliorates clinical symptoms and neuropathology (Pifarre et al. 2011). We have additionally shown that sildenafil administration from the initiation of EAE symptoms prevents further clinical deterioration by stimulating immunomodulatory and neuroprotective mechanisms. The observation of an increased number of axons presenting thinner than normal but compact myelin sheaths suggested a remyelinating effect of sildenafil treatment (Pifarre et al. 2014). In this work, we have confirmed the remyelinating potencial of cGMP-mediated pathways by using organotypic cerebellar cultures demyelinated with lysophosphatidylcholine (LPC) and have investigated potential mechanisms involved in this effect. Results show that incubation of demyelinated cerebellar cultures with sildenafil, the NO-dependent guanylyl cyclase (NO-GC) activator Bay 41-2272 or the glutamate receptor agonist N-metil-D-aspartate (NMDA), alone or in combination, significantly increases the amount of myelin sheath per axon area (remyelination index) analyzed by confocal imaging of double myelin basic protein (MBP) and neurofilament (NF200) staining and also augments the density of nodes of Ranvier, analyzed by caspr staining. The remyelinating effect of sildenafil is inhibited by the NO-GC inhibitor ODQ and by the NO synthase type II inhibitor L-N-nitroarginine, indicating the involvement of the endogenous NO-cGMP system. We have also investigated maturation of oligodendrocytes as a potential mechanism implicated in the remyelinating effect of sildenafil. Results from double immunostaining for MBP and the immature oligodendrocyte marker olig2 in cerebellar cultures showed that sildenafil treatment increases the number of olig2-/MBP+ cells, indicating that it promotes the final stage of oligodendrocyte maturation. Similar results were observed in spinal cord of EAE mice.

Pifarre et al. Acta Neuropathol 121:499-508, 2011. Pifarre et al. Exp Neurol 251:58-71, 2014.

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P-5. THE EFFECTS OF ASTROCYTE-TARGETED PRODUCTION OF EITHER IL6 OR IL10 IN REGENERATION AFTER FACIAL NERVE AXOTOMY ARE NOT RELATED TO NEUROPEPTIDE PRODUCTION

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Interleukins are a group of cytokines with an important role in the immune response modulation. Specifically, interleukin-10 (IL10) and interleukin-6 (IL6) are widely known to be involved in the regulation of neuroinflammation after a CNS challenge. Previous work of our group has shown differences in neuronal survival after facial nerve axotomy (FNA) in transgenic mice overexpressing either IL6 or IL10 under the GFAP promoter (GFAP-IL6Tg and GFAP-IL10Tg). Concretely, GFAP-IL6Tg mice showed a decrease in neuronal survival at 21 days post-injury (dpi) after FNA while GFAP-IL10Tg presented an increase in motor neuron number in the facial nucleus respect to wild-type (WT) mice. A more extended study about axonal regeneration provided early evidence of an influence of these cytokines in this process. Importantly, at 42 dpi GFAP-IL6Tg still showed a decrease in neuronal survival compared to WT. Besides, a retrograde tracer at mouse vibrissae revealed lower regeneration rate to the target muscle in GFAP-IL6Tg compared to WT, while GFAP-IL10Tg showed no differences. However, the expression of molecules related to nerve regeneration has not been explored. The objective of this work is to study the relation between neuropeptides and axonal regeneration differences induced by the local production of IL10 or IL6 after FNA. With this aim, FNA was performed in GFAP-IL6Tg, GFAP-IL10Tg and WT. Cryostat sections from 3 to 42 dpi were obtained and immunohistochemistry for galanin and calcitonin gene-related peptide (CGRP) detection was performed. Finally, their staining was quantified and analysed. Our observations showed a slight increase in CGRP at 21 dpi in GFAP-IL6Tg respect to WT. Regarding galanin expression, both GFAP-IL10Tg and GFAP-IL6Tg presented an augmented production at 35 dpi. In conclusion, the slight changes observed in galanin and CGRP in the facial nucleus are not related to the effect of IL6 or IL10 local production in long-term regeneration after FNA.

P-6. MICROGLIAL CULTURES DERIVED FROM ADULT HUMAN MONOCYTES: CHARACTERIZATION AND RESPONSES TO A-SYNUCLEIN

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The importance of glial cells in CNS function is indisputable. In the case of microglial cells their role in human CNS disease appears to be particularly relevant. Unfortunately, the possibility to culture human microglial cells is limited at present and most of our knowledge on microglial biology was obtained in rodent cultures. Recently, a protocol to differentiate adult human monocytes into microglia-like cells has been reported (Ohgidani et al, 2014, Scientific Reports).

The aim of this study has been to reproduce this protocol, to evaluate ways to improve it and to characterize the responses of human microglia-like cells to α-synuclein. Adult human monocytes were isolated by Histopaque density gradient centrifugation, differentiated into microglia-like cells with GM-CSF and IL-34 and used after 14 DIV. Nineteen independent cultures were prepared (01/2015-05/2016). Modifications regarding seeding, medium changes and differentiation conditions were compared to the original protocol.The protocol of culturing microglia-like cells from adult human monocytes was successfully reproduced. Inter- and intra-experimental variability in final cell density and cell morphology was observed. Variations in seeding, medium changes and differentiation conditions did not affect cell density and morphology and therefore the original protocol was applied.Immunocytochemistry showed weak internalization of monomeric α-synuclein by human microglia-like cells. In contrast, aged α-synuclein was markedly internalized in a concentration-dependent manner at 6 and 24 hours. ELISA experiments showed α-synuclein-induced production of the proinflammatory cytokines TNFα and IL-6, but not of IL-1β. Surprisingly, monomeric α-synuclein was a more potent inducer of TNFα and IL-6 production than aged α-synuclein.

In conclusion, microglia-like cell cultures from adult human monocytes are an interesting tool to study human microglia biology. Their responses to α-synuclein may offer clues to the role of these cells in Parkinson's disease. Efforts need to be made to minimize and understand the sources of inter- and intra-experimental variability.

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P-7. ASTROCYTIC CHLORIDE CHANNEL ACTIVATION DEFECT IN MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC), a rare leukodistrophy characterized by myelin and astrocyte vacuolization, is caused by mutations in either MLC1 or GLIALCAM genes. GlialCAM is a necessary subunit of MLC1 protein, indispensable for the correct targeting of MLC1 to astrocyte junctions. GlialCAM has also been identified as a ClC-2 chloride channel auxiliary subunit. In vitro, GlialCAM is able to target ClC-2 channel to cell-cell junctions, as well as changes its currents from inwardly rectifying to opening the channel at all voltages. However, in vivo studies show ClC-2 current modification by GlialCAM only in oligodendrocytes, but not in astrocytes. Similarly, ClC-2 chloride currents recorded in primary cultured astrocytes, where MLC1 and GlialCAM are expressed, are inwardly rectifying, and ClC-2 is not interacting with GlialCAM. Thus, the relationship between GlialCAM and ClC-2 in astrocytes is not clear, as neither it is the role of the chloride channel in astrocyte physiology. In this work we found that in cultured rat astrocytes incubated in the presence of high potassium levels, which mimic neuronal activity, the subcellular localization and functional properties of ClC-2 are changed. We provide evidences that under this conditions the three proteins MLC1, GlialCAM and ClC-2 may form a ternary complex necessary for this change in ClC-2 localization and function, and prove that this mechanism is impaired in astrocytes where MLC1 is absent, thus suggesting a possible mechanism involved in the pathophysiology of MLC disease. Our results suggest that the regulation of ClC-2 activity by GlialCAM in astrocytes may be needed to compensate the high levels of potassium in depolarizing conditions, which may help in the process of potassium siphoning by glial cells.

P-8. PRELIMINARY STUDY ON THE EFFECTS OF NANOPOROUS SILICA PARTICLES ON MICROGLIA

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Nanoporous silica particles (nPSPs) are designed as a drug delivery tool for the stabilization and controlled, local and long-lasting release of large molecules. Their good physical stability and chemical inertness combined with their exceptionally large pore volume make them a promising drug delivery system. Our groups participate in a multidisciplinary study aimed at developing novel particle-based therapeutic approaches for the treatment of amyotrophic lateral sclerosis. Our main hypothesis is that nPSPs can be effective tools for local delivery of neuroprotective peptides at the site of compromised neurons. Before being used, it is necessary to know their effects on CNS cells.

The aim of this study has been to analyze the responses of microglia to nPSPs. To this end, nPSPs of 5-10µm diameter were labelled with Rhodamine B and applied to various types of primary glial cultures: murine mixed glial, murine microglial and human microglia-like cells. Morphological changes, viability, particle phagocytosis and cytokine production were analyzed. No changes in cell morphology or viability induced by nPSPs were observed. nPSPs were phagocytosed by microglia in the three preparations tested. Internalized particles were observed one hour after nPSPs addition. The number of cells with particles and of particles per cell peaked at 24h and remained high after 14 days suggesting lack of degradation of nPSPs by microglia. Finally, nPSPs did not induce IL-6 production by mixed glial cultures. This preliminary study shows that nPSPs do not induce marked changes in viability and morphology or a pro-inflammatory reaction in microglia. Phagocytosis of nPSPs by microglia may be a drawback to the use of nPSPs as a drug delivery system in the CNS. Given the anti-inflammatory nature of CNS parenchyma it is likely that microglial phagocytosis of nPSPs is lower in vivo than in vitro. In vivo studies are needed to answer this question.

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P-9. ROLE OF INTERLEUKIN 6 AND INTERLEUKIN 10 IN NERVE REGENERATION AFTER FACIAL NERVE AXOTOMY

CANCELED

P-10. EFFECTS OF AXONAL REGENERATION ON INFLAMMATION AFTER SCIATIC NERVE INJURY IN MICE

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Injury to the nervous system results in degeneration of axons extending distally from the injury site and their myelin sheaths, a process known as Wallerian degeneration. The inflammatory response that takes place during Wallerian degeneration is important for axonal regeneration as immune cells phagocytose the degenerating myelin sheats, which are enriched in molecules that exhibit axonal growth. There are, however, striking differences in the Wallerian degeneration that occurs in the peripheral nervous system (PNS) and central nervous system (CNS). Among them, the failure of CNS to resolve the inflammatory response following lesion, as well as the cytotoxic contribution of macrophages to the damaged CNS. Whether these differences are a consequence of the incapacity of CNS axons to regenerate after lesion is currently unknown. Herein, we assessed whether axonal outgrowth is a key event that controls the resolution of inflammation and the functional phenotype of macrophages in the nervous system. We characterized, by combining fluorescence-activated cell cytometry (FACS) and histological analysis, the dynamics of the inflammatory response and macrophage polarization when the sciatic nerve is crushed and axonal regeneration occurs, and when the sciatic nerve is cut and not repaired to impede axonal outgrowth. We found that axonal regeneration is a key event that induces late stage of macrophages clearance in the injured nerve, but ir does not participate in switching the functional phenotype of macrophages from a proinflammatory to an inflammatory phenotype. This study provide new insights in the mechanisms that modulate the inflammatory response in the injured nervous system.

P-11. CHARACTERIZATION OF A TRANSGENIC MOUSE MODEL OVEREXPRESSING TNFA IN MYELINATING SCHWANN CELLS

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TNFα has been implicated in the pathogenesis of peripheral neuropathy, among other inflammatory demyelinating diseases and neuropathic pain. TNFα is a pro-inflammatory cytokine that act at several stages in the demyelination process. It is produced by Schwann cells (SCs) in the peripheral nervous system after nerve injury and released into the local environment which attracts and activates macrophages to contributing to Wallerian degeneration. In vivo studies demonstrated a local inflammation in the sciatic nerve of rats after injection of TNFα, followed by demyelination and axonal degeneration. Furthermore, the application of TNFα resulted in acute mechanical hiperalgesia, and TNFα is postulated as a biomarker for painful changes after nerve injury. With the aim to characterize TNFα effects in neuropathic pain and in peripheral neuropathy as well as to develop efficient gene therapy strategies, a transgenic mouse model overexpressing TNFα cDNA under the peripheral myelin protein P0 promoter was generated. Here we characterized the overexpression of TNFα in myelinated SCs at different stages of myelination showing that high levels of TNF-α in sciatic nerve correlated with increased mRNA levels of TNF-α and leads to the downregulation of the major PNS myelin proteins both at mRNA and protein levels, correlating with loss of structured myelin in the sciatic nerve and an increase in p75NTR, a marker for immature and non-myelinated SCs. Iba1 staining showed high levels of macrophage infiltration at both sciatic and spinal cord tissues. Stress conditions were induced by crush surgery in the sciatic nerve after which recovery and subsequent remyelination were delayed in the transgenic mice, as assessed by the Sciatic Functional Index and electrophysiological tests, thus correlating molecular and phenotypic changes in this mouse line. On the other hand, mechanical and thermal nociception seemed to be unaltered. This model could be helpful in the characterization of the role TNF-α.

P-12. IL-13 ADMINISTRATION FAVORS MICROGLIA AND MACROPHAGES TO ADOPT AN M2-LIKE PHENOTYPE AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) elicits an inflammatory response that comprises mainly microglia and macrophages. These cells contribute directly or indirectly to tissue damage and functional loss; however, they can also promote repair. These paradoxically conflicting roles of microglia and macrophages depend on their polarization state: In response to interferon gamma or lipopolysaccharide, these cells undergo M1 polarization. Contrary, upon interleukin 4 (IL-4) or interleukin 13 (IL-13) stimulation, macrophages and microglia acquire M2 polarization. M1 macrophages and microglia release high levels of pro-inflammatory cytokines. These compounds induce damage in healthy neighboring cells and are associated with cell loss and secondary damage after SCI. Contrary, M2 macrophages release anti-inflammatory cytokines and are involved in tissue repair and remodeling events. Macrophages and microglia display predominantly M1 markers after SCI, whereas the expression of M2 markers is limited. To get insights into the mechanisms that impede microglia and macrophages to acquire an M2-like phenotype after SCI, we found that the expression of IL-13, one of the most important M2 polarizing factors, is detected at very low levels in the contused spinal cord. We therefore hypothesized that inefficient induction of IL-13 expression after SCI favors microglia and macrophages to remain in a M1-like state. We first evaluated the expression of IL-13 receptor (IL-13Rα1) and found it is induced in microglia and macrophages following SCI. Interestingly, we observed that microglia and macrophages induce the expression of the M2 marker Arg1 upon IL-13 administration into the lesion site. Moreover, IL-13 administration reduced the expression of the M1 markers, iNOS and CD16/32. These results provide evidence that low levels of IL-13 after SCI hamper microglia and macrophages to acquire an M2-like activation state. Further studies are needed to elucidate whether the redirection of microglia and macrophages polarization by IL-13 minimize tissue damage and functional deficits after SCI.

P-13. EFFECT OF LOCAL CNS PRODUCTION OF EITHER IL6 OR IL10 IN NERVE REGENERATION AFTER PERIPHERAL NERVE INJURY

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Facial nerve axotomy (FNA) is a model of peripheral nerve injury mainly characterized by retrograde degeneration of motor neurons in the facial nucleus (FN), axonal regeneration and glial activation. Previous studies from our group with transgenic mice producing either IL6 or IL10 under the GFAP promoter (GFAP-IL6Tg and GFAP-IL10Tg mice) have shown that these cytokines are able to modulate the CNS response after FNA. Significantly, a decrease of neuronal survival was observed in GFAP-IL6Tg mice at 21 days post-injury (dpi) compared to wild-type (WT) mice, whereas GFAP-IL10Tg mice showed a neuronal survival increase. Nevertheless, the effect of both cytokines in axonal regeneration has not been explored yet. The aim of this study is to investigate the effect of IL6 or IL10 production in axonal regeneration after FNA. Therefore, FNA was performed in GFAP-IL6Tg, GFAP-IL10Tg and WT adult mice. Neuronal survival was analysed on cryostat sections stained with toluidine blue at 42 dpi, and axonal regeneration was assessed after injecting FluoroGold (FG), a retrograde fluorescent marker, at 35 dpi in the whiskerpads, and evaluating labelled neurons at 42 dpi. In addition, the regeneration-associated molecule CD44 was studied from 3 to 28 dpi. The results showed no differences in neuronal survival at 42 dpi when comparing transgenic GFAP-IL6Tg or GFAP-IL10Tg respect to WT mice. In contrast, FG analysis revealed less regenerating motor neurons targeting the vibrissae in GFAP-IL6Tg mice compared to WT. Finally, a decrease of CD44 was observed at all time-points in GFAP-IL6Tg mice respect to WT, while in GFAP-IL10Tg CD44 expression increased at 14 and 28 dpi. In conclusion, our findings indicate that local CNS production of IL6 or IL10 do not affect long-term neuron survival but may influence nerve regeneration after FNA.

P-14. EFFECT OF TOXIC AGENTS ON GLIAL CELLS: CONTRIBUTION TO NEUROTOXICITY IN PARKINSON'S DISEASE

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Neuroinflammation, in which microglial cells are mainly involved, is a common process in neurodegenerative diseases. Although reactive microglia are detected in the brain of Parkinson's disease (PD) patients, their role in the etiology and progress of the pathology still remains uncertain. Both genetic and environmental factors are postulated to contribute to the pathogenesis of PD, and epidemiological studies suggest that pesticide exposure is a risk factor for the disease. Some chemical agents which are inhibitors of the mitochondrial respiratory chain, such as MPTP/MPP+ and the pesticide rotenone, are involved in the development of PD. However, although the neurotoxic effect of this kind of compounds has been widely reported using in vivo and in vitro experimental approaches, their direct effect on glial cells remains poorly characterized. The aim of the present work was to study the effect of MPP+ and rotenone on glial activation and on mechanisms involved on the control of microglia inflammatory phenotype, such as the CD200-CD200R1 ligand-receptor pair.

Mouse primary glial cell cultures (mixed glial and microglial cultures) were treated with MPP+ or rotenone in the absence and the presence of a pro-inflammatory stimulus (LPS+IFN-γ). We determined metabolic activity, cell viability, cell morphology and the expression of effector molecules which participate in the inflammatory response. CD200 and CD200R1 expression was evaluated in the same experimental conditions. In addition, the neurotoxicity of these treatments was assessed on mouse primary mesencephalic cell cultures.

MPP+ and rotenone induced morphological and functional changes in glial cells. These compounds inhibited the metabolic activity in mixed glial cultures. In addition, they inhibited the inflammatory response in activated glial cells and altered CD200 and CD200R1 expression. Consequently, glial cells exposed to MPP+ or rotenone display an altered functionality that results in an impaired immune response, which may contribute to their reported dopaminergic neurotoxicity in PD.

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DESENVOLUPAMENT, NEUROGÈNESI I CÈL.LULES MARE

P-15. MOLECULAR MECHANISMS REGULATING WIRING SPECIFICITY

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A fundamental requirement in the assembly of neural circuits is that neurons establish synaptic connections with their appropriate partners. In many systems, this involves extension into a particular synaptic layer, and selection of the appropriate partner among all the cells in the layer. Virtually nothing is known about the mechanisms governing the establishment of specific connections in any system. Our hypothesis is that the molecular differences that exist between neuronal subtypes, with similar developmental origin and function, contribute to their distinct connectivity. We study the differential layer selection of the closely related Drosophila R7 and R8 photoreceptors. Each eye contains 750 R7 and 750 R8 cells, and the entire population of each subtype proceeds synchronously to their respective final synaptic layer during pupal development. Taking advantage of such precise coordination, we have profiled the R7 and R8 transcriptomes right before this final extension. Our bioinformatics analysis has identified differentially expressed genes between the R7 and the R8. We have focused on 229 R8 enriched genes and performed an RNAi screen. Out of 186 genes analyzed we have identified 44 candidate genes showing layer selection defects. We are currently confirming our findings using mutant alleles. We expect that characterization of these genes contributes to the understanding of the molecular mechanisms regulating R7 and R8 differential layer selection.

P-16. PRONGF/P75/SORTILIN SIGNALING: POTENTIAL TARGETS TO RECOVER NEUROGENESIS IN ALZHEIMER'S DISEASE

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The adult neurogenesis in humans has significant implications in memory formation and cognitive functions. Adult-born neurons generated in hippocampus are able to stabilize synaptic connections and mediate neuronal plasticity. The induction of neurogenesis can reverse cognitive dysfunction in animal models of Alzheimer's disease. Previous studies have shown that the levels of pro-NGF are increased in a stage-dependent manner in human hippocampus and entorhinal cortex affected by Alzheimer's disease. Also, the proNGF receptor, p75NTR, is expressed not only in mature neurons but also in mitotic cells from mouse hippocampus. To assess the role of these signaling molecules in the adult neurogenesis, immunofluorescence analyses were performed to detect proNGF, p75NTR, sortilin and differentiation or synaptic markers in the mouse model of Alzheimer's disease, in the hippocampal neurospheres cultured of p75NTR deficient mice, and also in the hippocampal preparations from Alzheimer's disease-affected human brains. The results showed that adult newborn cells from both human and animal models, express increased levels of p75NTR and sortilin in Alzheimer's disease. These neurons can respond to proNGF by apoptosis and/or dedifferentiation. Consequently, increased pro-NGF in the adult hippocampal brain, may be responsible for the decrease of neurogenesis. Disruption of proNGF/p75/sortilin signaling would be a relevant target in regenerative therapies for Alzheimer's disease.

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P-17. NCAM2 ROLE IN NEURITOGENESIS

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Neural cell adhesion molecule 2 (Ncam/Ocam/Rncam) is a cell adhesion molecule (CAM), homologous to Ncam1, that belongs to the immunoglobulin (Ig) superfamily. Ncam2 ectodomain is composed by five immunoglobulin domains and two fibronectin type III homology domains. Moreover, Ncam2 presents two different isoforms: a transmembrane isoform (Ncam2.1) and a glycosylphosphatidylinositol (GPI) anchor isoform (Ncam2.2). In mammals, Ncam2 is expressed in several tissues but the highest expression is found in the brain; its expression starts during embryonic stages and it is maintained in the adult. NCAM2 function has been extensively investigated in the olfactory system where it plays an important role in fasciculation and neurite outgrowth. Its function may be explained for its homophilic and heterophilic interactions. However, Ncam2 partners remain unknown.Ncam2 is also expressed in the hippocampus during development and in the adult. Nevertheless, Ncam2 function in this region still poor understood. In order to analyze Ncam2 interactome, we used a mass spectrometry approach. We find out more than a 100 proteins that interact with Ncam2. These proteins include cytoskeleton components and regulatory molecules that could mediate Ncam2 function in neuritogenesis and branching. The role of Ncam2 in neuritogenesis was studied in detail in in vitro hippocampal cultures. Our data suggest that Ncam2 is essential for neurite outgrowth, dendritic arborization and axon formation during brain development.

P-18. STUDY OF THE ROLE OF EBF-1 IN MEDIUM SPINY NEURONS SPECIFICATION AND MATURATION

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Ebf-1/Olf-1 is a helix-loop-helix transcription factor previously described to be strongly expressed in the Lateral and Medial Ganglionic Eminences from early embryonic stages (E11) until postnatal stages (P15). Its disruption affects the proper pattern of expression of genes involved in striatal neurogenesis. However, little is known about the mechanism of action of Ebf-1.

In this work we further characterize Ebf-1 expression and its specific roles in striatal development. We demonstrate that Ebf-1+ cells are generated through the first and second striatal neurogenic waves (E12.5-E16.5). Ebf-1 is mainly expressed in the mantle zone but it is also detected in few proliferative cells at the germinal zone. Ebf-1 is not only expressed by mature neurons but also by astrocytes, although it did not co-localize with oligodendrocyte markers. At the neuronal level of expression we showed that Ebf-1, which is expressed by Ctip2+ Medium Spiny Neurons (MSNs), is not expressed by DRD2+ MSNs, revealing specific functions for Ebf-1 in DRD1 neurons. Interestingly, we also found some Ebf-1+ cells in the cortex that might be Medial Ganglionic Eminence-derived since it co-localize with some interneuron markers *in vitro* such as ChAT, Calretinin and PV. Analysis of Ebf-1 knockout mice showed a reduction in striatal size quantified at E18.5 pointing out a possible implication in neuronal differentiation and/or maturation. In fact, a decrease in the total number of Ctip2+ cells was found in E18.5 Ebf-1 KO mice. Furthermore, Ebf-1 overexpression in striatal proliferating progenitors induced a decrease of Ki67+ cells. In addition, its overexpression in striatal cultures induced an increase of the calbindin+ neurons along with a reduction of the number of Nkx2.1+ cells. Overall, our findings indicate potential roles of Ebf-1 in cell cycle exit and cell specification and maturation during striatal development.

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P-19. EFFECTS OF THE SPINAL CORD INJURY ENVIRONMENT ON THE DIFFERENTIATION CAPACITY OF HUMAN NEURAL STEM CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Spinal cord injury (SCI) causes loss of neural functions below the level of the lesion due to interruption of spinal pathways and secondary neurodegenerative processes. The transplant of neural stem sells (NSCs) is a promising approach for the repair of SCI. Reprogramming of adult somatic cells into induced pluripotent stem cells (iPSC) is expected to provide an autologous source of iPSC-derived NSCs avoiding the immune response as well as ethical issues. However, there is still limited information on the behavior and differentiation pattern of transplanted iPSC-derived NSCs within the damaged spinal cord. We transplanted iPSC-derived NSCs, obtained from adult human somatic cells, to rats at 0 or 7 days after SCI, and evaluated motor evoked potentials and locomotion of the animals. We histologically analyzed engraftment, proliferation and differentiation of the iPSC-derived NSCs and the spared tissue in the spinal cords at 7, 21 and 63 days post-transplant. Both transplanted groups showed a late decline in functional recovery compared to vehicle-injected groups. Histology showed proliferation of transplanted cells within the tissue, forming a cell mass. Most grafted cells differentiated to neural and astroglial lineages, but not to oligodendrocytes. Some cells remained still undifferentiated and proliferating at final time points. The proinflammatory ambiance of the injured spinal cord induced proliferation of the grafted cells. Therefore, iPSC-derived NSCs cells have a potential risk for transplantation. New approaches are needed to promote and guide cell differentiation, as well as reducing their tumorigenicity once the cells are transplanted at the lesion site.

P-20. HUMAN PLURIPOTENT STEM CELLS ACHIEVE A MATURE TELENCEPHALIC NEURONAL PHENOTYPE AFTER 37 DAYS OF IN VITRO DIFFERENTIATION

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Human pluripotent stem cells (hPSCs) are a powerful tool for studying human neurodevelopment in health and disease, drug screening and cell therapy. However, precise protocols to differentiate specific neurons of interest are needed. Here we present a detailed description of feeder-free neuronal differentiation of hPSCs towards functional telencephalic neurons in 37 days in vitro (DIV).

Gene expression was analyzed at different stages (0, 8, 12, 16, 23 and 37 DIV) by OpenArray. We also assessed differentiation by immunocytochemistry against pluripotent, neuroectodermal, neural progenitors and neuronal markers. HPSCs showed a neural fate induction after 8 DIV through the application of SMAD and WNT inhibitors. At 16 DIV hPSC-derived neuroepithelial cells were obtained and committed to pallial (PAX6+) and subpallial (DLX+ and EBF1+) telencephalic progenitors. Application of a defined medium from 16 DIV onwards induced further maturation and the generation of a complex neuronal network. This network was composed of 95% post-mitotic MAP2B+ neurons, of which GABAergic neurons represented the main neuronal sub-type including a sub-population of striatal projection neurons.Neuronal maturation was further analyzed by synaptic protein expression profiling, and network activity was analyzed using a high-resolution single-cell calcium imaging technique at 37 DIV. Neurons expressed the synaptic proteins SNAP-25, Syntaxin, synaptotagmin, synpatophysin-1, PSD-95 and NMDA receptors. Furthermore, calcium imaging showed that 84% of the neurons elicited spontaneous spikes-like events with different patterns of activity being observed including calcium oscillations, single- and burst of spikes.

In conclusion, we present a novel rapid feeder-free protocol for the differentiation of hPSCs into mature and physiologically active telencephalic neurons, which can be used for human disease modelling, drug screening and cell therapy applications.

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MALALTIES NEURODEGENERATIVES I

P-21. LACK OF STRIATAL-ENRICHED TYROSINE PHOSPHATASE IMPROVES MOTOR AND COGNITIVE PHENOTYPE IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expansion of a CAG repeat in the huntingtin (htt) gene, which results in an aberrant form of the protein (mhtt). This leads to motor and cognitive impairments associated to hippocampal and corticostriatal alterations. The molecular mechanisms behind the selective neuronal dysfunction are not clear yet, but it has been proposed that an imbalance between phosphatases and kinases activity could be a key factor. One of these phosphatases is striatal-enriched tyrosine phosphatase (STEP), a brain-specific tyrosine phosphatase that opposes the development of synaptic strengthening leading to aberrant synaptic function. Previously, we reported that STEP activity is decreased in the striatum, hippocampus and cortex of R6/1 mouse model of HD and demonstrated a neuroprotective role of STEP against excitotoxicity in HD. Through the generation of a double mutant of R6/1 and STEPKO mice, we explored the effects of the lack of STEP in this mouse model. We found that genetic deletion of STEP improved motor and cognitive dysfunction present in R6/1 mice. The absence of STEP was accompanied by increased pERK1/2 levels in hippocampus and striatum. We also observed a decrease in DARPP-32 levels and in the number of large mhtt aggregates in the striatum. Together, these data demonstrate that lack of STEP in R6/1 mice delays motor and cognitive deficits suggesting that reduction of STEP in HD mouse models could be a compensatory mechanism.

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P-22. 7,8 DIHYDROXYFLAVONE AMELIORATES COGNITIVE AND MOTOR DEFICITS IN A HUNTINGTON'S DISEASE MOUSE MODEL THROUGH SPECIFIC ACTIVATION OF THE PLCΓ1 PATHWAY

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Huntington's disease (HD) is a fatal neurodegenerative disease caused by an expanded CAG trinucleotide repeat in the gene encoding the protein huntingtin, it presents with motor, cognitive and psychiatric disorders; HD displays reduced levels of striatal brain-derived neurotrophic factor (BDNF); thus BDNF activation through its receptor TrkB has therapeutic potential. 7,8-Dihydroxyflavone (7,8-DHF) was described as a TrkB agonist in several in vitro and in vivo models of neuro-degenerative diseases including HD but its precise TrkB activation profile needs further investigation.

AIMS: Test 7,8 DHF effect on cognitive and motor deficits on the R6/1 mouse model of HD. Elucidate the striatal TrkB activation profile of 7,8 DHF. Identify pleiotropic properties of 7,8 DHF useful in HD

METHODS: 7,8-DHF treatment (5 mg/kg daily from 8 to 20 weeks) of R6/1 mice tested for motor (rotarod) and cognitive (NORT) deficits; primary striatal cultures, immunocytochemistry, Ca++ Imaging.

RESULTS: 7,8 DHF delays the onset of motor deficits in R6/1 mice; reversed the inability to perform correctly the (NORT) at 15 weeks; pathological and biochemical analyses of treated mice revealed improved levels of Enkephalin in striatum, and prevention of striatal volume loss upon treatment. There was a $TrkB^{Y816}$ but not $TrkB^{Y515}$ phosphorylation recovery in striatum. Treatment in primary cultures confirmed the selective phosphorylation of Y816 residues in addition to morphological and functional improvements, different from controls treated with BDNF.

CONCLUSIONS: Our results suggest 7,8 DHF has therapeutic potential for HD but also that 7,8 DHF has differential effects from BDNF that should be further investigated.

P-23. REGULATION OF THE TRANSCRIPTION FACTOR NURR1 BY NEURONAL ACTIVITY IN HIPPOCAMPAL CULTURES

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Synaptic dysfunction is widely believed to precede neuronal loss in Alzheimer's disease (AD). Emerging evidences suggest that the impairment in synaptic transmission might occur through changes in the mechanisms that control AMPA receptors (AMPAR) gene expression, which are regulated by BDNF. It has been reported that BDNF is a target gene of the nuclear receptor related protein 1 (Nurr1) in glutamatergic neurons. Nurr1 is a member of the orphan nuclear receptor subfamily 4 of transcription factors activated by neuronal activity. Although it is well established that Nurr1 gene is essential for the differentiation and maintenance of meso-diencephalic dopaminergic neurons, novel functions for this gene have been recently proposed. Our working hypothesis is that a reduction in Nurr1 transcriptional activity could be responsible for the synaptic failure observed at early stages of AD. To properly characterize eventual changes in Nurr1 activity, we have explored the mechanisms underlying its expression in mice hippocampal cultures. During the first days in vitro (DIV), Nurr1 expression increases until 7 DIV, decreasing afterwards when neurons achieve maturity. To study the activity-dependent regulation in mature neurons, we have used 16 DIV cultures. When cells were treated with 4-AP or bicuculline, we observed a strong increase in Nurr1 levels. This increase is dependent on calcium entry through ionotropic glutamate receptors. Previous data reported that Nurr1 is regulated by the CREB signaling pathway including the involvement of its specific coactivator CRTC1. CRTC1 activation is mediated by the calcium-dependent phosphatase calcineurin. Accordingly, we observed that activity-dependent Nurr1 induction was dependent on CRTC1/CREB. Interestingly, we also found that Nurr1 induction depends on calcineurin. Finally, our results also show that BDNF and AMPAR (GluA1 subunit) expression is dependent on Nurr1. Altogether, these findings raise the possibility that Nurr1 could be a key transcription factor involved in activity-dependent changes in synaptic function.

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P-24. IMMUNOMODULATORY AND NEUROPROTECTIVE EFFECTS OF SILDENAFIL FOLLOWING BETA- AMYLOID ELEVATION

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Abeta-plaques are a pathological hallmark of Alzheimer's disease (AD). Activated glial cells are found surrounding plaques both in humans and in AD-mouse models. However, the role of glia around Abeta-plaques is still a matter of debate. In the presence of Abeta, glial cells become activated and secrete inflammatory mediators that have been related to disease severity. However, glial cells can also restrict plaque formation by Abeta phagocytosis and/or degradation via Aï ¢-degrading enzymes, suggesting a neuroprotective role of these cells in AD.

Abeta has been found to down-regulate the NO-cGMP-PKG pathway resulting in synaptic disruption. In agreement with this, elevation of cGMP levels by treatment with the cGMP-phosphodiesterase-5 inhibitor sildenafil produces a long-lasting amelioration of synaptic function and memory in a mouse model of Abeta-deposition, the APP/PS1 mouse. These effects are accompanied by a long-lasting reduction in Abeta-levels, by unknown mechanisms. We have now found that sildenafil reduces cortical microglial activation and potentiates hippocampal astroglial reactivity in the APP/PS1 mice. Furthermore, our in vitro studies suggest that sildenafil stimulates microglial phagocytosis of Abeta-oligomers. Interestingly, it has been described that sildenafil upregulates YM-1, a marker of macrophage/microglial M2 phenotype in the spinal cord of a mice model of mutiple sclerosis. In AD, this phenotype has been associated to Abeta-phagocytic activity. Thus, polarization of microglia to the M2 phenotype may also contribute to the sildenafil neuroprotective actions. On the other hand, it is known that oAbeta cause synaptic depression by inducing internalization of AMPA-receptor (AMPAR) subunits GluA1. The preliminary results show that PKG activation restores GluA1-levels in hippocampal neurons suggesting that the beneficial effects of sildenafil in AD mice may also involve regulation of synaptic AMPA-Rs at early stages of the disease. Our results indicate that sildenafil may contribute to delay the onset and/or progression of AD-related pathology through immunomodulatory and synaptic actions

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P-25. REGULATION OF NIGROSTRIATAL DOPAMINE NEUROTRANSMISSION BY GAMMA-SYNUCLEIN: DOWN- AND OVER-EXPRESSION MOUSE MODELS

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Synucleins are small, highly conserved proteins in vertebrates, especially abundant in neurons and typically enriched in presynaptic terminals. Mutations in α-synuclein gene have been reported in families susceptible to inherited forms of Parkinson's disease, but far less is known about other members of the synuclein family (β- and γ-synucleins), which have been implicated in the pathophysiology of neurodegenerative diseases and several synucleinopathies. Here, we examined the distribution and cellular localization of γ-synuclein in the mouse brain using in situ hybridization and immunohistochemistry procedures, and we evaluated the functional role of γ-synuclein in nigrostriatal dopamine (DA) neurotransmission in vivo. We used two different models with: 1) down-regulated γ-synuclein expression specifically in DA neurons of substantia nigra compacta/ventral tegmental area (SNc/VTA) by small interfering RNA molecules (siRNA), or 2) over-expressed γ-synuclein in SNc/VTA DA neurons using AAV10-CMV-mouse-γ-synuclein vector. In C57BL/6J control mice, abundant levels of γ-synuclein mRNA were found in the monoaminergic nuclei including SNc, VTA, raphe nuclei and locus coeruleus, as well as in the habenular nuclei. Double in situ hybridization and immunohistochemistry showed a specific colocalization of γ-synuclein in TH-positive DA neurons. Unilateral infusion of three siRNA sequences targeting γ-synuclein (4.2 nmol) decreased γ-synuclein mRNA levels in DA neurons of SNpc (50% and 80% of aCSF-treated mice 24h and 72h post-infusion, respectively). Furthermore, γ-synuclein suppression displayed an enhanced striatal DA tone using intracerebral microdialysis, which is reflected in higher extracellular DA levels in siRNA-treated mice during local veratridine (50 µM), nomifensine (1-10-50 µM) and amphetamine (1-10-100 µM) administrations and lower levels with quinpirole (10 µM). Meanwhile, γ-synuclein over-expression led to lower extracellular DA levels during nomifensine (1-10-50 µM) administration. In conclusion, these results confirm that γ-synuclein is a negative regulator of DA neurotransmission and suggest that siRNA-induced γ-synuclein suppression in midbrain dopaminergic neurons may lead to new therapies for synucleinopathies.

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P-26. ROLE OF NEWLY IDENTIFIED VOLUME-ACTIVATED CHLORIDE CHANNELS IN AN OCULAR NEURODEGENERATIVE DISEASE

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Glaucoma is a group of chronic, neurodegenerative diseases that affects the optic nerve. The main risk factor associated with the development of glaucoma is the elevated intraocular pressure (IOP). Trabecular meshwork (TM) cells actively filter and thus modulate aqueous humor (AH) outflow and in turn, intraocular pressure (IOP). Malfunction of these ocular structures causes, in a sequential way: decrease in the AH outflow, IOP increase, degeneration of retinal ganglion cells and a progressive and irreversible visual field loss.

In previous studies, we characterized the key role of Volume-Regulated Anion Channels (VRAC) in the TM cell volume regulation as well as AH outflow facility. However, the molecular identity of VRAC at that time was unknown. The recent identification of five isoforms of Leucine-Rich Repeat-Containing 8 (LRRC8A-E) proteins as essential components of $Cl_{(swell)}$ currents mediated by VRAC, opens the field to elucidate whether VRAC is involved in glaucoma by studying LRRC8A-E at transcriptional, translational or functional level.

Our results show significant changes in the expression and functional activity of VRAC when human cell lines derived from glaucomatous (HTM-3) and normal TM (HTM-5) are compared. mRNA of all isoforms of LRRC8(A-E) can be detected in both HTM cell lines but glaucomatous cells (HTM-3) show diminished levels of expression compared to normal cells (HTM-5), while the glaucoma biomarker ELAM-1 is highly increased. Western blot analysis also demonstrate that LRRC8A total protein levels as well as LRRC8A protein on the cell surface (assessed by surface biotinylation) are lower in glaucomatous HTM-3 cells than in normal HTM-5 cells. In agreement with molecular data, hypotonicity-activated $Cl_{(swell)}$ currents are significantly reduced and show a delayed activation in glaucomatous cells, suggesting an impairment of VRAC channel activity and TM cell volume regulation in glaucoma pathophysiology.

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P-27. HIGH FAT DIET COGNITIVE DISTURBANCES IN AGED MICE ARE MEDIATED BY INFLAMMATION AND WNT PATHWAY ALTERATION: NEUROPROTECTIVE MECHANISMS OF RESVERATROL

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Oxidative stress and inflammation are on the basis of ageing theories. High fat diet (HF) is demonstrated to induce a number of cellular processes impairment as reduced metabolic rate, radical oxygen species (ROS) produced by mitochondria and finally inflammation process. All together that can impact in the health of individuals. We studied the effect of HF feeding in aged C57/BL6 (24 months) regarding cognition and determining molecular changes underlying the loss of cognitive capabilities and focus on new possibilities in resveratrol beneficial effect in the oldest.

We studied cognitive function by Novel Object Recognition test (NORT), biochemical proinflammatory cytokines by RT-PCR and components of Wnt pathway by Western blot.

After 8 weeks of HF, old mice presented a significant loss in memory in reference to control and also to young mice. Animals fed with resveratrol (160mg/kg/day) are protected against cognitive loss induced by HF. Hippocampus was studied for molecular changes underlying HF and resveratrol effects.

TNF-α, Cxcl-10, IL-1, IL-6 and Ccl3 gene expression increased in HF diet mice hippocampus, demonstrating that HF induced an inflammatory process in brain. Resveratrol reduced their expression both in controls and in HF diet groups. HF diet induced a decrease in Wnt activation, producing an increase of beta-catenin phosphorylation. This marks beta-catenin degradation and reduced the transcription of neuroprotective factors. C57/BL6 fed with resveratrol showed a lower level on GSK-3β phosphorylation in Ser9 residue and then a reduction in the degree of β-catenin phosphorylation, indicating that supplementation with resveratrol recover Wnt pathway activity in older mice, delivering a neuroprotective effect that can explain the better cognitive performance in NORT in animals fed with this polyphenol with antioxidant properties. Finally, partial correlation analysis determined the robust relationship among the cognitive evaluation, inflammaging and Wnt pathway activation levels and the influence of resveratrol restoring HF induced damage in aged mice.

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P-28. ROLE OF IL-6 TRANS-SIGNALING IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most commonly diagnosed dementia. The main hallmarks of AD are a progressive loss of cognitive functions and the presence of extracellular deposits of aggregated β-Amyloid and intracellular deposits of hyperphosphorylated tau protein. These features lead to a prominent neuroinflammation, being interleukin-6 one of the critical cytokines. For signaling, IL-6 binds to a membrane receptor (mIL-6R), then, the complex associates with gp130. Whereas the expression of gp130 is ubiquitous, the expression of mIL-6R is restricted to some tissues, thereby only cells with mIL-6R would respond to IL-6. Interestingly, a soluble form of IL-6R (sIL-6R) exists naturally, and it is demonstrated that this form can activate gp130 in the presence of IL-6. This process has been called trans-signaling and it is specifically inhibited by the soluble form of gp130, sgp130.

To clarify the role of IL-6 trans-signaling in Alzheimer's disease, we use a mouse model of AD, 3xTg-AD, which simultaneously co-produces the specific inhibitor of IL-6 trans-signaling, human sgp130-Fc, in astrocytes. In this study, we will characterize the model, both behaviorally and neuropathologically. Here, I present a part of the study, including weight monitoring, survival and behavioural characterization, using Open-field, Hole-board, Elevated-Plus Maze and Morris Water Maze before the formation of β-amyloid plaques (4-5 months of age). We will also analyze these same parameters after the formation of β-amyloid plaques (13-14 months of age).

Additionally, molecular and immunohistochemistry analyses will be done to give further insight into the role of IL-6 trans-signaling in Alzheimer's disease.

La Marató de TV3

P-29. LICOCHALCONE A, AN INHIBITOR OF JNK1, A DRUG TO PREVENT CYTOTOXIC AND NEUROINFLAMMATORY DAMAGE IN A TEMPORAL LOBULAR EPILEPSY MICE MODEL

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Licochalcone A (Lic-A), a molecule derived from the plant Glycyrrhiza inflata, has shown antimalarial, anticancer, antibacterial and antiviral properties. On this work, we demonstrate the role of Lic-A as an antiepileptic drug and how this molecule is able to prevent neuroinflammatory and cytotoxic damage through the inhibition of the c-jun N-terminal kinase (JNK) pathway, specifically the isoform JNK1. Using a model of temporal lobular epilepsy (TLE) based on the administration of kainic acid (KA), it was studied how the inhibition protected adult wild-type mice. Animals were administered with Lic-A previous to the KA injection and were sacrificed 3, 24 and 72 h later in order to obtain hippocampal tissue for protein, mRNA, stains, immunofluorescence and immunohistochemical studies. Lic-A administration caused a significant reduction on phosphorylation levels of total JNK protein. Also, brain inflammatory responses associated to both astrocytes and microglia were reduced, along with protein levels of tumour necrosis factor α (TNF- α). Neuronal death was evaluated through FluoroJade B and those animals treated with Lic-A previous to KA injection showed no neurodegeneration. Pro-apoptotic and anti-apoptotic signals were also affected: Bcl-2 like protein 4 (BAX), Bcl-2 like protein 11 (BIM) and cleaved α-spectrin showed reduced expression, along with a significant increase in B-cell lymphoma 2 (Bcl-2) protein. Ultimately, the neurogenesis response after KA administration was evaluated using several markers like protein kinase B (AKT), cAMP response element-binding (CREB) and NESTIN, and those animals that had been administrated with Lic-A showed a reduced response. Consequently, it can be concluded that the inhibition of JNK1 by Lic-A could be an effective drug in the prevention of neuroinflammation, apoptosis and neurogenesis derived of epilepsy in this TLE mice model.

Centro de Investigación Biomédica en Red, Enfermedades Neurodegenerativas (CIBERNED)

P-30. NEUREGULIN-1 PROMOTES FUNCTIONAL IMPROVEMENT BY ENHANCING COLLATERAL SPROUTING IN SOD1^{G93A} ALS MICE AND AFTER PARTIAL MUSCLE DENERVATION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of motoneurons, which is preceded by loss of neuromuscular connections in a "dying back" process. Neuregulin-1 (Nrg1) is a neurotrophic factor essential for the development and maintenance of neuromuscular junctions, and Nrg1 receptor ErbB4 loss-of-function mutations have been reported as causative for ALS. Our main goal was to investigate the role of Nrg1 type I (Nrg1-I) in SOD1^{G93A} mice muscles. We overexpressed Nrg1-I by means of an adeno-associated viral (AAV) vector, and investigated its effect by means of neurophysiological techniques assessing neuromuscular function, as well as molecular approaches (RT-PCR, western blot, immunohistochemetry, ELISA) to determine the mechanisms underlying Nrg1-I action. AAV-Nrg1-I intramuscular administration promoted motor axon collateral sprouting by acting on terminal Schwann cells, preventing denervation of the injected muscles through Akt and ERK1/2 pathways. We further used a model of muscle partial denervation by transecting the L4 spinal nerve. AAV-Nrg1-I intramuscular injection enhanced muscle reinnervation by collateral sprouting, whereas administration of lapatinib (ErbB receptor inhibitor) completely blocked it. We demonstrated that Nrg1-I plays a crucial role in the collateral reinnervation process, opening a new window for developing novel ALS therapies for functional recovery rather than preservation.

P-31. ROLE OF CRTC1 IN SYNAPTIC MORPHOLOGY DURING NEURODEGENERATION

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Dendritic spines are small protrusions in the dendrites that represent the main post-synaptic site of excitatory glutamatergic synapses. Spine morphology varies in response to synaptic activity and long-term potentiation. In Alzheimer's disease (AD) and other neurological disorders, learning and memory impairments correlate with dendritic spine number and morphology changes, but the molecular mechanisms underlying these changes are largely unknown. Interestingly, dysfunction of the CREB-regulated transcription coactivator-1 (CRTC1) is associated with altered gene transcription and memory deficits in AD mouse models. In this project we have characterized for the first time the morphology of synapses in the hippocampus of a presenilin (PS) conditional knockout (PS cDKO) mice that displays classical features of neurodegeneration occurring in AD. We have also studied the role of CRTC1 overexpression in the regulation of spine number and morphology in WT and PS cDKO mice. Our results indicate that PS cDKO mice exhibit dysmorphic dendritic spines that present excessive enlargement of the heads and elongation of the necks. CRTC1 overexpression partially reverts these alterations by shortening spine necks. Contrarily, in WT mice, CRTC1 modifies the spine heads and increases the length of the necks. These findings reveal a differential role of CRTC1 depending on physiological or neurodegenerative conditions. Importantly, the modifications in spine morphology induced by CRTC1 overexpression may correlate with the learning and memory improvements in WT and PS cDKO mice after CRTC1 injection previously reported by our group. Therefore, our results suggest that CRTC1 may represent a new strategy to ameliorate synaptic pathology during neurodegeneration.

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P-32. MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE: ROLE OF THE MITOCHONDRIAL PROTEIN IMPORT

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Mitochondrial dysfunction has long been implicated in the pathogenesis of Parkinson's disease (PD). However, the molecular mechanisms linking mitochondrial dysfunction with cell death are still under investigation. Mitochondria contain approximately 1,500 different proteins, 99% of which are encoded by the nuclear genome. Nuclearly-encoded mitochondrial proteins are imported into mitochondria by mitochondrial membrane translocases: translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM). Protein import represents a dynamically regulated process that varies in response to oxidative stress or aging. Here we have investigated in vitro and in vivo whether complex I inhibition impair the mitochondrial protein import systems.

In the in vivo MPTP-induced mouse model of PD, complex I inhibition induces a decrease in the protein levels of mitochondrial translocases TOM20 and TIM23 before the occurrence of cell death, suggesting that deficient protein import is an early event in PD.In a dopaminergic cell line model, complex I inhibition is associated not only with a downregulation of TOM20 and TIM23 protein levels, but also with a partial blockage of protein import into mitochondria. Interestingly, complex I inhibition induces an enrichment in detergent-insoluble mitochondrial proteins, with a specific increase of a ATPaseV-subunit suggesting that complex I inhibition impairs the proper folding or degradation of mitochondrial proteins, resulting in its accumulation.Moreover, complex I inhibition leads to deficient mitochondrial respiration, enhanced production of reactive oxygen species, loss of mitochondrial membrane potential and neuronal cell death. Importantly, overexpression of TIM23 and/or TOM20 complex subunits attenuates complex I induced defects and complex I induced cell death, thus demonstrating the deficient mitochondrial protein import is instrumental in complex I inhibition-induced neuronal death. Collectively, these findings provide evidence for a link between mitochondrial protein import dysfunction and neuronal loss, revealing a possible new therapeutic target for PD.

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P-33. EARLY CHANGES INDUCED BY A-SYNUCLEIN IN MONOAMINE NEURONS: NEW THERAPEUTIC STRATEGIES FOR PARKINSON'S DISEASE

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Pathological changes in end-state of Parkinson's disease (PD) are well characterized. However, there is an urgent need to identify early functional changes to develop new therapeutic strategies stopping the development of the illness. α-Synuclein is a protein accumulating in the brain of PD patients. To understand the sequence of events occurring in PD, we generated a mouse model overexpressing wild-type human-α-synuclein in dopamine-DA or serotonin-5-HT neurons of substantia nigra (SN) and raphe nuclei (RN), respectively. We used an adeno-associated virus type-5 (AAV5)-α-synuclein vector, unilaterally injected in SN or RN. AAV5-α-synuclein mice showed increased human-α-synuclein mRNA levels in the ipsilateral SN and RN (278 and 290% of sham mice, respectively, 8-week post-infection), without changes of endogenous α-synuclein. Immunohistochemistry analysis revealed increased human-α-synuclein and phospho-α-synuclein levels; but, the mice did not display loss of tyrosine hydroxylase-(TH)-positive or tryptophan hydroxylase-(TPH2)-positive neurons. However, impairments in DA or 5-HT release paralleled with development of α-synuclein-positive axonal swelling in forebrain were found. Veratridine perfusion (50µM) decreased DA and 5-HT release in the striatum of AAV5-α-synuclein mice. Likewise, nomifensine or citalopram (1-10-50µM) reduced striatal DA or 5-HT levels, respectively, in AAV5-α-synuclein versus sham mice. Moreover, these mice displayed motor impairments and depressive-like behaviors. In order to reduce the expression of human α-Syn, we administrated intracerebro-ventricularly an indatraline-conjugated antisense oligonucleotide (ASO1337, 30 and 100 µg/day) during 28 days using osmotic minipumps. ASO1337 sequence was designed to selectively target human α-Syn in the monoaminergic neurons. Data indicated a significant reduction dose-dependent of human α-Syn mRNA levels in the SNc. Moreover, intranasal ASO1337 treatment (30 days) also decreased human α-Syn expression in the midbrain nuclei of over-expressed mice. Synaptic DA and 5-HT dysfunctions and axonopathy would thus be the hallmark of early-stage of PD and suggest that ASO targeted α-synuclein in monoamine neurons may lead to new therapies for PD.

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P-34. NEUROPROTECTIVE EFFECT OF 11B-HSD1 INHIBITION THROUGH AUTOPHAGY ACTIVATION IN SAMP8 MOUSE MODEL

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Alzheimer's disease (AD) is the most common cause of dementia and represents a progressive brain disorder, characterized by loss of cognitive functioning (memory impairments) and behavioral abilities. Aging represents a major risk factor for neurodegenerative diseases, as AD. Elevated glucocorticoids (GCs) exposure is widely accepted as a key factor in age-related cognitive decline. In fact, high levels of GCs have been found in elderly who exhibit learning and memory impairments and correlates with greater hippocampal atrophy. Recent studies have demonstrated that aged mice with cognitive deficits show increased 11β-Hydroxysteroid dehydrogenase (HSD1) expression in the hippocampus and forebrain, and that overexpression of this enzyme displays a similar premature memory decline. It has been reported that 11β-HSD1 inhibition ameliorated memory in AD animal models.

In the present work, we determine the neuroprotective effect of a potent 11β-HSD1 inhibitor (RL-118, IC50 = 29 nM), featuring a pyrrolidine polycyclic scaffold, administrated during four weeks (21mpk) to Senescence Accelerated Mouse-Prone 8 (SAMP8) mice, that displays a phenotype of accelerated aging with AD trends and that is widely used as an excellent rodent model of cognitive dysfunction.We investigated effects on cognition through novel object recognition test (NORT) and object location test (OLT), and neuroprotection by synaptic, inflammatory and autophagy markers through Western blotting and qPCR. Results showed a significant prevention in cognitive capabilities. Moreover, reduced loss in synaptic markers as PSD95 protein levels and diminution of proinflammatory cytokines expression indicate a cognitive impairment preventive action of the 11β-HSD1 inhibitor. These positive effects are due to changes in the autophagy process, because increased Beclin 1 and p62 expressions, jointly with reduced activation of mTOR were demonstrated.

Taken together, 11β-HSD1 inhibition is able to prevent cognitive decline during the aging process by reducing inflammation and activating the authophagy process, improving the cell capability to remove misfolded proteins.

P-35. THE EFFECTS OF DEXIBUPROFEN IN APP/PS MICE AS FAMILIAL MODEL OF ALZHEIMER´S DISEASE

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Alzheimer´s disease (AD) is the most common form of progressive dementia, which affects 5-10% of the world´s population. This disease is characterized by increased levels of extracellular Beta-Amyloid (βA) plaques and intracellular neurofibrillary tangles composed by hyperphosphorylated TAU. It has been demonstrated that these processes are tightly linked to neuroinflammation in which the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) can reduce the risk and progression of AD; nevertheless, their mechanism remains still unknown. For this reason, the main objective of this study was to evaluate the effects of NSAID dexibuprofen (DXI), which is the active isomer of ibuprofen, in 6-month-old female APP/PS1 mice as familial model of AD.

DXI was orally administered at dose of 20mg·kg-1·d-1 for 3 months until their sacrifice. As expected, the results showed a significant increase of NFKβ, TNFα, nNOS and iNOS hippocampus protein levels, all of them involved in inflammatory response, in APP/PS1 mice compared to WT mice. By contrast, animals treated with DXI showed a significant decrease of these levels. Moreover, Il6 mRNA level was significantly decreased in APP/PS1 DXI group. In line with that, the analyses of microglia and astrocytes showed an increased reactivity in APP/PS1 vs. WT and a reduction in treated mice. In addition, the number of βA plaques was analyzed showing a significant decreases in APP/PS1 DXI mice. Several molecules involved in TAU phosphorylation were studied such as c-ABL and CABLES and different phosphorylations of TAU. Their protein levels showed a significant increase in APP/PS1 compared to WT; nevertheless, they were decreased in DXI group. Besides, protein levels related to memory processes showed a significant increase in treated mice vs. non-treated mice. In conclusion, our findings demonstrate that DXI could be an effective drug in the prevention of AD together with others.

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P-36. APOLIPOPROTEIN E ISOFORMS DIFFERENTIALLY MODULATE ASTROCYTE EXCITABILITY

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Apolipoproteins (ApoE) are cholesterol carriers mainly secreted by astrocytes in the brain that exist in three alleles in humans: E2, E3 and E4. Despite the fact that ApoE4 is one of the most important genetic risk factor in late-onset Alzheimer´s disease (LOAD), its pathogenic mechanism remains unknown. Since calcium signaling—a hallmark of astrocyte excitability—are dysregulated in LOAD, the objective of this study is to determine the effect of the ApoE allele on calcium homeostasis in astrocytes. As a model, we have used immortalized astrocytes in which the murine ApoE has been replaced by human ApoE isoforms. We have discovered that ATP, Noradrenaline or Acetylcholine induced peak-and-plateau cytosolic calcium increases, which were smaller and shorter in ApoE3 cells compare to ApoE4 cells. The direct measurement of organellar calcium fluxes and the pharmacological manipulation of calcium channels show differences between calcium mobilization in ApoE4- and ApoE3 expressing astrocytes, being NAADP-mediated calcium release from lysosomes the most relevant one. Moreover, the different calcium response of ApoE3 and ApoE4 cells is independent of ApoE level expression or lipidation status, because, ApoE silencing in ApoE3-astrocytes and treatment with bexarotene, a drug that increases ABCA1 (responsible of the extracellular lipidation of ApoE) have no effect on ATP-induced calcium responses. Finally, we have found out that ApoE4, unlike ApoE3, are not responsive to the extracellular environment. In particular, absence of FBS (fetal bovine serum) or hydroxycholesterol in the extracellular media up-regulates the magnitude of the cytosolic calcium peak induced by ATP in ApoE3 cells. We conclude that ApoE regulates calcium signaling in astrocytes and the expression of ApoE3 or ApoE4 alleles is determinant for such regulation. Ongoing work is aimed to explore the underpinnings, consequences and therapeutic implications of these findings.

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P-37. DENSE-CORE VESICLE SECRETORY PROTEINS IN THE BRAIN AND CEREBROSPINAL FLUID OF ALZHEIMER'S DISEASE PATIENTS

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Alzheimer disease (AD) is the most prevalent neurodegenerative disorder characterized by the occurrence of amyloid plaques and neurofibrillary tangles and aberrant functions of neuronal and glial transmitters. Peptidic transmitters are targeted, processed and stored in dense-core vesicles (DCV). In response to a physiological signal, release of DCV-contained neuropeptides, growth factors and secretory machinery components is triggered by regulated exocytosis. Classical members of the chromogranin/secretogranin family have been analyzed in the normal and AD brain. However, other abundant DCV components such as the non-classical granin SgIII and the enzyme convertases CPE, PC1/3 and PC2 have been poorly studied. These proteins exert key roles in sorting, aggregation and processing of peptidic transmitters in the regulated secretory pathway. Here, we evaluated alterations of these DCV-contained proteins in the brain and cerebrospinal fluid (CSF) of AD patients. In the control human neocortex and hippocampus, DCV proteins were located in neuronal cell bodies and processes and in astroglial cells, in a lesser extent. In AD, dystrophic neurites and reactive astrocytes surrounding amyloid-beta plaques showed a differential immunolabeling for DCV proteins. Moreover, altered levels of DCV components were differentially observed in the CSF of AD patients. For instance, reduced levels of the unprocessed SgIII form were detected in AD patients and positively correlated with the potential AD biomarker Cystatin C. Because DCV molecular components are essential in the processing and secretion of neuropeptides and neurotrophins, their participation in the pathophysiology of AD may be suggested.

P-38. EXPLORING BRAIN GENE EXPRESSION CHANGES FOLLOWING ISCHEMIC STROKE THROUGH MICROARRAYS

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Ischemic stroke is a complex neurovascular disease that ranks as a leading cause of death and disability worldwide. Many molecular and signaling pathways altered after stroke are known, although a broad analysis of gene expression changes in human brain after stroke has not been conducted so far. Our aim was to identify and to verify these changes at brain level and to interpret the results in a biological context.

Total RNA was isolated from flash-frozen brain samples from the infarct core (IC) and healthy contralateral (CL) areas of 11 patients who died due to ischemic stroke. Affymetrix Human Transcriptome Arrays were employed to explore gene expression profiles in 6 patients, comparing ICvsCL samples. Data obtained was evaluated using the R libraries developed at the Bioconductor project and explored through Ingenuity Pathways Analysis software. 723 genes were differentially expressed ($p<0.05$ after adjustment by False Discovery Rate), with genes up-regulated in IC being involved in the immune response and, conversely, those down-regulated genes being related to neuronal function. 23 top candidate genes, based on the highest and lowest logarithmic fold change, were selected to be replicated in samples from the remainder 5 patients by means of qRT-PCR using TaqMan probes. The same expression patterns than in the microarrays were found for all the candidate genes, with 2 up-regulated genes showing statistical significance ($p<0.05$).

Our findings provide some interesting candidates to be explored as potential neuroprotective therapeutic targets in the context of stroke. Moreover, our ongoing integration of this gene expression data with protein levels information obtained by mass-spectrometry in the same samples will help us to build the integrome of brain after stroke and to find flagship molecules for the understanding and management of this disease.

NEUROTRANSMISSIÓ

PÒSTERS 1

P-39. TWO-PHOTON ACTIVATION OF FREELY DIFFUSIBLE ALLOSTERIC PHOTOSWITCHES

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Optical tools for both probing and controlling the activity of neurons with light have seen a great expansion in the last few years, since the spatiotemporal precision offered by light matches with the spatiotemporal complexity of cell-to-cell communication in the brain. An example of this precision is pulsed illumination with near-infrared (NIR) lasers, which is widely used for imaging activity deep in 3D and scattering tissues such as the brain. NIR light is used to define a micrometric volume where effector molecules such as neurotransmitters are optically activated, for instance allowing the uncaging of neurotransmitters in a volume the size of a dendritic spine.

Recently, we developed and characterized alloswitch-1, an azobenzene-based optical probe for allosterically switching on/off a metabotropic glutamate receptor (mGlu5) with violet/green light (Pittolo et al. 2014). We aimed at activating alloswitch-1 also with NIR light through two-photon (2P) lasers. The 2P excitation (2PE) of azobenzene-based molecules was previously demonstrated by our group (Izquierdo-Serra et al. 2014), but was not obvious in the case of the freely-diffusible alloswitch-1, since 2PE of alloswitch-1 to the cis isoform would limit the optical activation of mGlu5 in a small volume, but diffusion of the trans isoforms from outside the volume of 2PE could immediately replace the outgoing cis-isoforms and block the signaling downstream of mGlu5. Here, we demonstrate the 2PE of alloswitch-1 and some of its derivatives. This is the first evidence that optical control of freely diffusible allosteric modulators of neuronal receptors is possible with NIR light. The 2PE of alloswitch-1 has an axial resolution as good as 10 µm, which opens up interesting possibilities for its use in intact tissues and in vivo with unprecedented tissue depth and spatial resolution.

P-40. COGNITIVE IMPAIRMENT INDUCED BY DELTA9-TETRAHYDROCANNABINOL OCCURS THROUGH HETEROMERS BETWEEN CANNABINOID CB_1 AND SEROTONIN 5-HT_{2A} RECEPTORS

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Activation of cannabinoid CB_1 receptors (CB_1R) by delta9-tetrahydrocannabinol (THC) produces a variety of negative effects with major consequences in cannabis users that constitute important drawbacks for the use of cannabinoids as therapeutic agents. For this reason there is a tremendous medical interest in harnessing the beneficial effects of THC. Behavioral studies carried out in mice lacking 5-HT_{2A} receptors (5-$HT_{2A}R$) revealed a remarkable 5-$HT_{2A}R$-dependent dissociation in the beneficial antinociceptive effects of THC and its detrimental amnesic properties. We found that specific effects of THC, such as memory deficits, anxiolytic-like effects, and social interaction are under the control of 5-$HT_{2A}R$, but not its acute hypolocomotor, hypothermic, anxiogenic and anti-nociceptive effects. In biochemical studies, we show that CB_1R and 5-$HT_{2A}R$ form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment. Remarkably, our functional data shows that co-stimulation of both receptors by agonists reduces cell signaling, antagonist binding to one receptor blocks signaling of the interacting receptor, and heteromer formation leads to a switch in G-protein coupling for 5-$HT_{2A}R$ from Gq to Gi proteins. Synthetic peptides with the sequence of transmembrane helices 5 and 6 of CB_1R, fused to a cell-penetrating peptide, were able to disrupt receptor heteromerization in vivo leading to a selective abrogation of memory impairments caused by exposure to THC. These data reveal a novel molecular mechanism for the functional interaction between CB_1R and 5-$HT_{2A}R$ mediating cognitive impairment. CB_1R-5-$HT_{2A}R$ heteromers are thus good targets to dissociate the cognitive deficits induced by THC from its beneficial antinociceptive properties.

P-41. DYRK1A, A NOVEL REGULATOR OF NMDA RECEPTORS: IMPLICATIONS FOR DOWN SYNDROME AND ALZHEIMER'S DISEASE

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N-Methyl-D-Aspartate glutamate receptors (NMDARs) play a pivotal role in synaptic plasticity processes and molecular mechanisms of cognition. Under certain conditions, NMDAR activation can induce neuronal dysfunction and excitotoxicity, which are associated to synaptic dysfunctions present in Alzheimer's disease (AD) and Down syndrome (DS). We have previously shown that DYRK1A kinase (a DS candidate gene product) overexpression promoted an increased surface expression of GluN2A subunit-containing NMDARs, together with a prolonged decay of NMDA-induced calcium currents. In this study, we identified the specific effect of DYRK1A on GluN2A subunit. DYRK1A directly phosphorylates GluN2A at serine residue 1048 (S1048), within its intracellular domain. Functionally, DYRK1A-mediated phosphorylation reduces GluN1/GluN2A internalization rate, increases their surface expression and potentiates NMDA-evoked current density. Considering the regulatory effects of phosphorylation on NMDAR surface density, subcellular distribution and biophysical properties, these data support a role for DYRK1A as a direct regulator of GluN1/GluN2A subtype of NMDARs. Notably, DYRK1A upregulation has been described in both DS and AD murine models. In agreement, we detected an increase in GluN2A-Ser1048 phosphorylation in adult brain of DS murine models. This approach might help in understanding the role of NMDAR phosphorylation in the initial stages of these related synaptopathies, with potential therapeutic applications.

P-42.BDNF/TrkB/PKC SIGNALING MODULATED BY SYNAPTIC ACTIVITY CONTROLS THE PHOSPHORYLATION OF THE EXOCYTOTIC PROTEINS MUNC18-1 AND SNAP-25 AT THE ADULT NEUROMUSCULAR JUNCTION.

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In the synapses, several signaling pathways coordinate pre-, post-synaptic responses and associated glial cell. The relation between these signaling pathways modulate the voltage dependent calcium channels (VDCC) and the ready releasable pool of synaptic vesicles leading to neurotransmitter release (Südhof et al., 2013). In addition, the final functional outcome of a synaptic contact can be built by the confluence of the metabotropic receptor-mediated signaling on intracellular protein kinases as PKC and PKA (reviewed by Tomas et al., 2014). Although the mechanism underlying neurotransmitter release has been extensively studied there is still not fully understood the molecular machinery of synaptic vesicle exocytosis. Therefore, the present study is aimed to know (1) how Munc18-1 and SNAP-25 phosphorylation are affected by synaptic activity at the neuromuscular junction, (2) how BDNF/TrkB signaling pathway affects Munc18-1 and SNAP-25 phosphorylation in an activity-dependent way and (3) how presynaptic nPKCε and cPKCβI regulate Munc18-1 and SNAP-25 phosphorylation. We performed immunohistochemistry and confocal techniques to evidence the presynaptic location of Munc18-1, nPKCε and cPKCβI in diaphragm muscle. To induce synaptic activity, we stimulated the phrenic nerve (1 Hz, 30min) with or without contraction (μ-conotoxin GIIIB was used to abolish muscle contraction). Specific inhibitory reagents were used to block nPKCε and cPKCβI activity and to modulate tyrosine kinase receptor B (TrkB): exogenous BDNF, anti-TrkB (clone 47/TrkB); εV1-2, nPKCε-specific translocation inhibitor peptide; εV1-3, cPKCβI specific translocation inhibitor peptide. Main results obtained from Western blot experiments showed a relationship of dependence between skeletal muscle contraction, TrkB signaling, presynaptic nPKCε and cPKCβI and Munc18-1 and SNAP-25 phosphorylation at the adult rat NMJ. Together, these results provide a mechanistic insight into how phosphorylation of these exocytotic proteins are regulated to achieve the extraordinary speed, precision, and plasticity of neurotransmission.

PÒSTERS SESSIÓ II

SISTEMES SENSORIAL I MOTOR I TERAPIES

P-43. PYRETHROIDS INHIBIT K_{2P} CHANNELS AND ACTIVATE SENSORY NEURONS: BASIS OF INSECTICIDE-INDUCED PARAESTHESIAS

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Pyrethroid insecticides are widely used for pest control, either in agriculture or in public health in humans, commonly as topical treatment for scabies and headlice. It is known that exposure to pyrethroid insecticides, such as permethrin or tetramethrin (TM), can cause sensory alterations in humans such as transient pain, burning, stinging sensations and paraesthesias. The main known effects of these insecticides are on sodium and chloride channels but other channels may contribute as well to the alterations reported. Given the role of two-pore domain potassium (K_{2P}) channels in modulating sensory neuron excitability and firing, both in physiological and pathological conditions, here we examined the effect of pyrethroid insecticides on TRESK and other K_{2P} channels mainly expressed in sensory neurons (TREK-1, TREK-2 and TRAAK). TRESK currents prevent excessive sensory neuron activation and a decrease in its expression or function results in neuronal hyperexcitability after injury or inflammation and in altered sensory perception.

Small dorsal root ganglion sensory neurons (mainly nociceptors) were depolarized and fired action potentials in response to TM. Calcium imaging experiments showed that a high percentage of TM-activated neurons also responded to agonists of TRPA1 and/or TRPV1, expressed in nociceptors. Intradermal TM injection in the mouse paw evoked nocifensive responses and caused mechanical allodynia, demonstrating that the effects seen on the nociceptors lead to painful behaviors. Whole-cell patch clamp recordings in TRESK, TREK-1, TREK-2 or TRAAK-transfected cells showed inhibitory effects of TM on these channels, being more pronounced for TRESK and TRAAK. Skin-nerve recordings showed activation of C-fibers in the saphenous nerve after TM application. In TRESK knockout mice, TM elicited enhanced painful responses, thus reinforcing the role of this channel in preventing excessive neuronal activation. Our results suggest that the painful effects of pyrethroids are partially mediated through inhibition of K_{2P} channels, which regulate sensory neuron excitability.

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P-44. ANIONIC PHOSPHOLIPIDS BIND TO AND MODULATE THE ACTIVITY OF HUMAN TRESK, A TWO-PORE DOMAIN POTASSIUM CHANNEL EXPRESSED IN SENSORY NEURONS

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The two-pore domain potassium channel TRESK is implicated in pain perception pathways and changes in channel expression and function alter sensory neuron excitability. TRESK has emerged as a therapeutic target for treatment of pain-related disorders.

The recent observation of TRESK activation by shear stress and membrane stretch led us to hypothesise that direct interactions between TRESK and membrane phospholipids could be a mechanism by which such physical factors influence channel activity.

To begin to test this hypothesis, we used an in silico approach to identify potential anionic phospholipid-binding sites in human and rat TRESK. This analysis revealed that both human and rat TRESK contain potential sites in the large cytoplasmic loop.

To determine whether these sites could bind to anionic phospholipids in vitro, we analysed the ability of the human and rat TRESK loops to bind to Folch Fraction multilamellar vesicles (MLVs), which are enriched in anionic phospholipids. We observed specific interaction of the human, but not the rat, TRESK loop portion with MLVs. Similar experiments using MLVs of defined lipid composition demonstrated that the human TRESK loop did not bind to MLVs composed solely of the neutral phospholipid, phosphatidylcholine (PC), whilst showing strong binding to liposomes containing mixtures of either phosphatidylserine (PS)/PC or PC/PS/phosphatidyl-inositol 4,5-bisphosphate (PIP_2). We have started to investigate the functional significance of this interaction in HEK293 cells transfected with human TRESK. Disruption of electrostatic lipid-TRESK interactions by Poly-lysine treatment inhibited TRESK currents, whilst subsequent application of Folch Fraction MLVs caused a significant activation of TRESK currents above basal levels. These results suggest the anionic phospholipid-TRESK interaction serves to positively modulate channel activity. Current experiments are focused on determining the specific role of the lipid-binding region identified from our in-vitro studies and whether the interaction plays a role in the modulation of channel activity by membrane stretch.

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P-45. TRESK CHANNELS REGULATE THE EXCITABILITY OF SENSORY NEURONS INVOLVED IN NON-HISTAMINERGIC ITCH

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TWIK-Related Spinal cord K^+ channel (TRESK), a background potassium channel of the K_2P family, has a main role in setting the resting membrane potential, modulating action potential firing and neuronal excitability. This channel is expressed in sensory neurons that detect noxious stimuli (nociceptors) and is involved in the production of painful sensations, but less is known about his role in itch sensations. A subset of nociceptive neurons that express specific transient receptor potential (TRPs) and Mas-related G protein-coupled receptors (Mrgprs) are activated by different pruritogens and mediate itch sensations.

Because TRESK is key in pain generation, we hypothesize that background K^+ channels specifically expressed in neurons that present receptors for pruritogenic mediators, are likely to modulate itch sensations and constitute a potential target for the treatment of chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma or different types of dermatitis.

Through bioinformatic analysis, we identified specific TRESK expression in subpopulations of sensory neurons involved in itch generation that contain receptors for chloroquine (MrgprA3), histamine (H1) or b-alanine (MrgprD). Different populations of primary cultured sensory neurons from both wild-type or TRESK knockout (KO) mice were activated by chloroquine (CQ) or histamine in calcium imaging experiments. Injection of histamine in the mouse cheek produced similar itch behavior in wild-type and KO mice. In contrast, CQ administration produced a larger effect in animals lacking TRESK compared to wild-type, which suggests that TRESK might be modulating the non-histaminergic itch pathway. In addition, co-injection of a TRESK activator (cloxyquine) with CQ in wild-type animals markedly reduced itch behavior. Our data indicate that TRESK is involved in the regulation of the excitability of a subset of sensory neurons mediating histaminergic-independent itch, which have been proposed to have a prominent role in chronic itch diseases, highlighting TRESK as a possible candidate for therapeutic intervention.

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P-46. CENTRAL MODULATION OF NEUROPATHIC PAIN AFTER PERIPHERAL NERVE INJURY INDUCED BY TREADMILL EXERCISE

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Neuropathic pain develops as a result of lesions or diseases damaging the somatosensory nervous system, pharmacological treatments currently available are inefficient. However recent studies demonstrate hypoalgesic effects of exercise after peripheral nerve injury. It is well known that cortical areas can modulate the ascending pain signals by serotonergic and noradrenergic projections to spinal cord, which can facilitate or inhibit the activity of spinal cord neurons. In this study we characterize changes in the expression of serotonin and noradrenalin receptors induced by exercise in the spinal cord, after peripheral nerve injury. We performed sciatic spared nerve injury (SNI) in rats as a model of painful neuropathy, 3 days post injury (dpi) they started the increasing-intensity treadmill training for 5 days until 7 dpi, and from day 10 till 14 dpi. We assessed the hyperalgesia by means of mechanical and thermal sensory tests, and studied the receptors expression in the dorsal horn of the spinal cord by immunohistochemistry. SNI reduced the alpha-1A and beta-2 expression and increased serotonin-2A expression in inhibitory interneurons. Treadmill significantly upregulated alpha-1A receptor in the lamina II and beta-2 receptor in the lamina III, suggesting that hypoalgesia may be mediated in part by these effects. However, the alpha-2A receptor may be not involved in the hypoalgesic effect of treadmill, since no significant change in its expression was observed. On the other side we found serotonin-2A receptor expression reduced by treadmill training in inhibitory interneurons suggesting that it may prevent the activation of serotonergic endogenous response to afferent pain.

P-47. A MOUSE MODEL OF CHRONIC OTOTOXICITY TO STUDY DAMAGE AND REPAIR PHENOMENA IN THE VESTIBULAR EPITHELIUM

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Vestibular toxicity in humans commonly results from chronic exposure, yet animal models to study this toxicity are usually based on acute or short-term repeated exposure paradigms. Thus, chronic ototoxicity models have been scarcely studied and, although the cellular and molecular mechanisms involved in chronic versus shorter exposure ototoxicity may be similar, they may also differ considerably. A recent study using rats exposed chronically to 3,3'-iminodipropionitrile (IDPN) has identified the dismantlement of the calyceal junctions between type I HCs and calyx endings as an early and reversible step in damage progression in the vestibular epithelium. This study examined whether these plasticity phenomena can be studied in a second species, the mouse. In several strain/sex combinations we recorded either limited vestibular toxicity or excessive systemic toxicity; however, exposure of male 129S1/SvImJ mice to 30 mM IDPN in the drinking water offered the desired model. As previously found in male Long-Evans rats, vestibular dysfunction, assessed weekly by a test battery, appeared progressively and reverted after the intoxication was terminated at 5 or 8 weeks of exposure. Confocal microscopy analysis of immunolabeled cristas from animals exposed for 5 or 8 weeks revealed a striking loss of proteins that characterize the calyceal junction, including the adhesion protein caspr1 and the extracellular matrix protein tenascin-C. Mislocalization of labeling for KCNQ4, a potassium channel also enriched in the calyceal junction area was also recorded. Recovery in immunolabeling for these proteins was observed after washout periods of 5 or 12 weeks following the 5 or 8 weeks of exposure, respectively. Therefore, this mouse model mimics the previously established rat model. Thus, the mouse can also be used to study the damage and repair phenomena occurring during chronic ototoxicity and recovery.

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P-48. (-)-EPIGALLOCATECHIN-3-GALLATE TREATMENT ON THE ASTROGLIOSIS MAY DIFFER IN PERIPHERAL AND CENTRAL NEUROPATHIC PAIN ANIMAL MODELS

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The present work aimed to study thermal hyperalgesia and gliosis in mice subjected to spinal cord contusion and chronic constriction injury of sciatic nerve, and their modulation by (-)-Epigallocatechin-3-gallate (EGCG) treatment. Balb-c mice were subjected to spinal cord contusion (SCI) using the weight drop technique (2g; 25 mm) or to a chronic constriction injury (CCI) of sciatic nerve, and treated with EGCG once a day during the first week after surgery. Then, thermal hyperalgesia was evaluated at 7 and 14 days post-surgery (dpo) using the Hargreave's test. At the end of the experimental period, spinal cord of each animal was removed and processed by immunohistochemical techniques to assess astroglial cells (GFAP). EGCG-treatment reduced thermal hyperalgesia both on central and peripheral neuropathic pain models. On the other hand, whereas EGCG-treatment reduced astrogliosis reactivity in SCI-treated mice, not significant reduction effects were found in CCI-treated mice. Two hypotheses may explain these results. Firstly, EGCG may have different cellular and/or molecular spinal cord targets that lead to thermal hyperalgesia reduction. On the other hand, both experimental models may share cellular/molecular EGCG-targets to alleviate neuropathic pain but the molecular/cellular pathways of astrogliosis may differ between models and only in SCI model may have EGCG targets to reduce also astrogliosis. Notwithstanding these findings, additional research will be necessary to further explore these hypotheses.

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P-49. EFFECTS OF EPIGALLOCATECHIN-3-GALLATE (EGCG) AND A VEGETAL POLYPHENOLIC EXTRACT TREATMENTS ON THERMAL HYPERALGESIA IN SWISS MICE SUBJECTED TO CHRONIC CONSTRICTION INJURY OF SCIATIC NERVE

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The International Association for the Study of Pain (IASP) defines peripheral neuropathic pain as pain caused by a lesion or disease of the peripheral somatosensory nervous system. Current neuropathic pain treatments only partially relieve neuropathic pain in 40-60% of patients. For this reason, new pharmacological strategies are being investigated. Among them, use of polyphenols is a promising one. Polyphenols constitute one of the most numerous and widely distributed groups of natural products in the plant kingdom. More than 8000 phenolic structures are currently known. Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols. It is well known that epigallocatechin-3-gallate (EGCG), the most abundant flavanol in green tea, reduces thermal hyperalgesia after chronic constriction injury (CCI) in mice and rats. In the present study we compared the effects of EGCG and a polyphenolic vegetal extract on thermal hyperalgesia in mice subjected to CCI. Female Swiss CD1 mice were anesthetized with sodium pentobarbital (50 mg/kg; i.p.), an incision was made in the right thigh, and the sciatic nerve was exposed. Two loose ligatures with 1 mm apart were then made around the nerve using the 6–0 poly-glycolic acid synthetic absorbable sutures. The incision was closed using 5–0 interrupted nylon sutures. Then, animals were treated with EGCG or with a polyphenolic vegetal extract at 10, 15 and 20 mg/kg, intraperitoneally daily during the first week post-surgery. At 0, 7, 14, 21 and 28 days post-surgery, thermal hyperalgesia was evaluated using the Hargreaves test . The results show that low doses of EGCG and polyphenolic extract reduce hyperalgesia at short term.

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P-50. EVALUATION OF MOTOR RECOVERY, THERMAL HYPERALGESIA, ANXIETY-LIKE RESPONSE AND DEPRESSION-LIKE BEHAVIOR AFTER SPINAL CORD INJURY IN MICE

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Neuropathic pain is present in 40% to 50% of spinal cord injured (SCI) patients, tending to become chronic, and associates with lower quality-of-life. Although few animal models are available, new models are needed to complete the array of chances to assay new therapeutic strategies to treat chronic neuropathic pain and its associated mood disorders. The present study aimed to characterize locomotor alterations and central neuropathic pain triggered by graded photochemical spinal cord injury (SCI) performed by Rose Bengal baths. Additionally, since patients with long-term pain often develop emotional disorders, this study was also designed to study behavioural disturbances associated to chronic pain. Exposed spinal cord (T8-T9) of Balb-c mice was bathed with 1.5% Rose Bengal for 10 min and illuminated at 95 kLux for 1 (RB1), 5 (RB5) or 10 (RB10) min. Thermal hyperalgesia (Hargreaves plantar test), locomotor activity (BMS and footprints) and anxiety-like behavior (open field and dark/like box) were evaluated at 1, 4 and 8 weeks post-surgery. At the end, depression-like behavior (forced swim) was assessed and the immunoreactivity of astroglia (GFAP) and peptidergic (CGRP) afferent fibers in dorsal horn was analyzed. Photochemical SCI caused a RB-time dependent hyperalgesia along 8 weeks and graded locomotor alterations. Animals showed also a gradual increased astrogliosis and a significant sprouting of CGRP afferent nerve fibres. Finally, whereas results suggest no significant differences in anxiety-like behavioural, RB5 and RB10 animals showed a significant depression-like behaviour at 8 weeks post-surgery. Photochemical SCI causes chronic thermal hyperalgesia and emotional disturbances in mice. The present animal model may be suitable for further pharmacological studies to treat thermal hyperalgesia and behavioural disturbances associated to SCI.

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P-51. A SPECIFIC TARGET AGAINST JNK1 OR JNK3 CAN REDUCE ABERRANT NEUROGENESIS INDUCED IN STATUS EPILEPTICUS

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Neurogenesis in the adult mammal brain occurs constitutively throughout postnatal life in two specific forebrain areas: the subventricular zone and the subgranular zone of the dentate gyrus. In the specific case of the dentate gyrus, during epileptogenesis, neurogenesis increase in human patients as well as in experimental animal models of epilepsy.

Kainic acid (KA) is a potent neurotoxic that is an agonist to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors. KA is able to induce a severe status epilepticus, typified by an increase in neurogenesis together with an ectopic localization and morphological abnormality of newborn neurons. The contribution of neurogenesis disturbances to epileptogenesis is still not clear. The c-Jun N-terminal kinase (JNK) pathway that belongs to the MAPKs family is involved in different processes such as neuronal death by excitotoxicity, cell proliferation, differentiation, neuronal migration and neuronal plasticity. Three JNK genes are expressed in humans; jnk1 and jnk2 are ubiquitously expressed, whereas expression of jnk3 is restricted to the CNS. The aim of the present study was to elucidate the role of (JNK) in the neurogenesis control after the induction of SE. In order to carry out this work, wild type (WT) and genetically modified mice for JNK (jnk1-/-, jnk2-/- and jnk3-/-) were treated with intraperitoneal injections of KA. Different markers of progenitor cells and immature migrating neurons were analyzed by immunohistochemistry. The results showed an increase of different cell markers in WT and in jnk2-/- mice. However, in jnk1-/-null mice, the number of progenitor and immature neurons which was higher than in the other genotypes, decreased after KA injections. In addition, in jnk3-/-, no alterations of proliferative cells were observed after KA treatment.

All these data evidence a specific control of JNK isoforms in neurogenesis and moreover support that JNK1 and JNK3 are a good target in the epileptogenic treatment.

P-52. IN VITRO ASSAY OF THE NEUROPROTECTIVE ROLE OF NEUREGULIN 1 AGAINST MOTONEURON DEATH

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motor neurons (MNs) of the brain, brainstem and spinal cord. Indeed, the MN death mechanism is still unclear. Recently it has been shown that Neuregulin 1 (NRG1) a trophic factor highly expressed in MNs and neuromuscular junctions was reduced in ALS patients and in ALS transgenic mice.

OBJECTIVE: The main goal is to evaluate the capacity of NRG1 to preserve the spinal MNs and also its ability to reduce the glial reactivity induced by chronic excitotoxicity, a pathogenic mechanism involved in ALS.

METHODOLOGY: An in vitro model based on postnatal rat spinal cord organotypic cultures exposed to the compound DL-threo-β-hydroxyaspartic acid (THA) is used to characterize the effect of NRG1 on MN survival and glial reactivity.

RESULTS: We found that addition of recombinant human NRG1 (rhNRG1) increased survival of spinal MNs overcoming the excitotoxic effects caused by THA.

CONCLUSION: NRG1 could be a potential therapeutic target to preserve the MN survival in ALS. Further experiments should elucidate whether NRG1 has an important role in the MN long-term survival and against other pathogenic mechanisms contributing to ALS.

P-53. SIRT1 AND SIRT2 DEACETYLASE ACTIVITY IS RELEVANT FOR MOTONEURON SURVIVAL AFTER HYPOGLOSSAL NERVE INJURY

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Over the last years, the enzyme family described as sirtuin (Sirt) has been related with neurological dysfunctions, in particular Sirt1 and Sirt2. However, the role of Sirt2 in neurodegeneration remains controversial since its inhibition has been proved neuroprotective in Huntington and Parkinson models while the opposite was observed using Amyotrophic Lateral Sclerosis models. Until now, nobody has addressed its role after trauma causing retrograde neurodegeneration of central nervous system neurons. We aimed to elucidate the contribution of Sirt 1 and Sirt2 in a trauma model of hypoglossal nerve axotomy that causes cell loss of motoneurons (MNs) by disconnection to the periphery. We used *in vitro* models based in endoplasmic reticulum stress induction with tunicamycin in cell line (NSC34) or in spinal cord organotypic cultures. We used pharmacological modulation of Sirt1 or Sirt2 activity to elucidate that Sirt1 activity is neuroprotective in both models. On the other hand Sirt2 activity has oposite role in both models. For the *in vivo*, we used pharmacological and genetic modulation to establish that increase of Sirt1 activity is beneficial for neuroprotection while inhibition of Sirt2 is detrimental for MN survival in this model. Finally, we demonstrated that the neuroprotective effect of sirtuins was not mediated through Hif 1α stability.

P-54. SERIAL BLOCK FACE-SCANNING ELECTRON MICROSCOPY: A TOOL FOR STUDYING DRUG DELIVERY STRATEGIES ACROSS THE BLOOD-BRAIN BARRIER

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We have recently conducted a series of experiments in order to gain insight the potential of the anti-transferrin receptor (TfR) 8D3 monoclonal antibody (MAb) to transport neurotherapeutics across the blood-brain barrier (BBB), conjugating this MAb to gold nanoparticles (AuNPs) that simulated an attached cargo and using transmission electron microscope (TEM) techniques (Cabezón et al., 2015). Although the obtained results provided relevant information about the intracellular trafficking mechanisms of the conjugate, the study was performed through two-dimensional (2D) image analysis, which comprises a series of limitations. A three-dimensional (3D) structural study could significantly contribute to have a more realistic insight regarding the transport of molecules across the BBB. In the present work we aim to determine whether the Serial block-face scanning electron microscope (SBF-SEM) technique, one of the recent approaches using the scanning electron microscope to acquire serial images and to three-dimensionally reconstruct large tissue regions, can be a useful tool to study the transcellular trafficking of the AuNPs conjugated to the 8D3. Therefore, we coated AuNPs with the 8D3 antibody, administered the 8D3-AuNP conjugates intravenously to mice and, 2,5 hours later, processed the mouse brain for SBF-SEM. We established and optimized the configuration settings suitable to perform 3D reconstructions of brain regions containing BBB segments and to characterize the AuNP-containing vesicles inside the blood cerebral endothelial cells. We determined that the SBF-SEM technique results adequate to study the trafficking of the 8D3-AuNPs across the BBB. Moreover, although the study highlights that the shape and the size of the vesicles containing the AuNPs are significantly more complex that previously predicted, it reinforces the trafficking model that we previously proposed in the 2D study.

P-55. INDUCTION OF CHRONIC CEREBRAL HYPOPERFUSION IN MICE

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The drainage of cerebrovascular and interstitial fluid might be altered in cerebrovascular diseases like stroke or chronic cerebral hypoperfusion as it seems to be driven by arterial pulsation (Hawkes *et al*. 2014). In order to study variations in drainage and clearance of brain fluids we have developed a mouse model of chronic cerebral hypoperfusion based on a previous study (Hattori *et al*. 2015) with modifications. It was reported that the model reproduces features of human subcortical vascular cognitive impairment, including multiple white matter infarcts with motor and cognitive deficits.

The model involves asymmetric CCA surgery (ACAS) by implantation of different devices to the right and left common carotid artery in C57BL/6J mice. On the right side, the placement of an ameroid constrictor made of hygroscopic casein material resulted in gradual occlusion of the vessel over 14 days. On the left side, a microcoil induced arterial stenosis. Changes in tissue perfusion and appearance of lesions were monitored with longitudinal MRI scans. Angiography showed the progressive arterial occlusion, in agreement with Arterial Spin Labelling data showing a significant reduction in cerebral blood flow at day 14 after the surgery, mainly on the right hemisphere. The T2-map led to determine the presence of ischemic lesions in the tissue. In order to assess motor and behavioral impairment some tests have been carried out (rotarod, hanging wire, Y-maze, NORT), but none of them was able to show significant neurological deficits. To visualize changes in fluid distribution, a fluorescent tracer was injected in the cisterna magna after 14 days of occlusion and the tissue is currently being analyzed by microscopy. With this study we set a mouse chronic brain hypoperfusion model with progressive onset that does not cause acute brain lesions.

Hawkes et al. *Brain Pathology*. 2014:2:396–403

Hattori et al. *The Journal of Neuroscience*. 2015.35(9):3915–3928

P-56. PERIODIC ACID-SCHIFF GRANULES

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Degenerative granular structures, frequently referred as “PAS granules” because of their positive staining with periodic acid-Schiff (PAS), appear progressively with age in the brains of a wide range of mouse strains, and are particularly noticeable in the senescence accelerated mouse prone 8 (SAMP8), which exhibits an accelerated ageing process. These round-to-ovoid structures, measuring up to 3µm, are formed by a degenerative procedure involving astrocytic processes and affecting their immediate neuropil. These structures are organized into clusters, each containing approximately 40-50 granules from a specific astrocyte. We have recently found that during the formation of PAS granules some epitopes emerge in them which should be considered as neo-epitopes because they are not present in healthy structures. We also observed that these neo-epitopes are recognized by natural antibodies which, as they are natural, are present in the blood plasma of mice from birth and without prior contact with external antigens. Moreover, as natural antibodies have been fixed by natural selection during evolution and are interspecific, the antibodies that recognize these neo-epitopes are also present in the plasma from other mammal species. We also determined that these natural antibodies are present as contaminants in a high percentage of commercial antibodies, producing numerous cases of false-positive immunostainings of PAS granules and causing the inconsistency of some of the theories about them. Thereafter, there is a need to revise these theories, and to clarify why the organism constitutively produces antibodies ready to react against the neo-epitopes that arise in the PAS granules.

P-57. NEW PERSPECTIVES ABOUT CORPORA AMYLACEA IN HUMAN BRAIN

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The presence of corpora amylacea within the normal aged brain and, to a greater extent, in a variety of neurological conditions including Alzheimer's disease, Parkinson's disease or Huntington's disease is well established. Corpora amylacea are spherical, oval or elongated glycoproteinaceous structures, ranging from less than 2 µm to about 50 µm in diameter, that accumulate mainly in periventricular and subpial regions of human brain. These corpora amylacea have been associated to degenerative granular structures that appear with age in mouse brain and are referred as "PAS granules" because of their positive staining with periodic acid-Schiff (PAS). We recently reported the presence of some neo-epitopes in PAS granules, and we also reported that these neo-epitopes can be recognized by natural antibodies that are present in mouse plasma and plasma from other mammal species. Taking all into account, we hypothesized that corpora amylacea on human brain could also contain neo-epitopes, and that human plasma, as well as those from other mammal species, could contain natural antibodies directed against them. We found that, effectively, corpora amylacea in brains from Alzheimer's disease donors and elderly donors contain neo-epitopes and that these neo-epitopes can be recognized by natural antibodies contained in human plasma and plasma from other species. We formally propose that corpora amylacea, and probably some other polyglucosan bodies, are waste containers in which deleterious or residual products are isolated to be thereafter eliminated through the action of the innate immune system. In any case, the presence of neo-epitopes on these structures and the existence of natural antibodies directed against them will become a new focus in the study of both age-related and degenerative brain processes.

P-58. LONG TERM EFFECTS OF RT-PA ON ZO-1, CLAUDIN-5 AND CAVEOLIN-1 LEVELS AFTER OXYGEN-GLUCOSE DEPRIVATION USING AN IN VITRO MODEL OF BLOOD BRAIN BARRIER

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Cerebrovascular homeostasis is maintained by the blood-brain barrier (BBB), which forms a highly selective permeability barrier between peripheral circulation and the central nervous system. In patients with ischemic stroke, recombinant tissue-type plasminogen activator (rt-PA) is used to accelerate recanalization of the occluded vessels. However, the increased risk of haemorrhagic transformation (HT) in ischemic injury associated with the administration of rt-PA, is an important limitation for the generalization of thrombolytic therapy. Despite the BBB disruption has been studied at early times post rt-PA administration showing a detrimental effect on thigh junction proteins, long time post-treatment effects are poorly understood. The aim of this study was to determine the effect of rt-PA on two tight junction proteins, ZO-1 and Claudin-5, and on Caveolin-1, that is involved in membrane traffic and permeability and therefore, a novel target in ischemia conditions. An in vitro BBB model performed with the bEnd3 immortalized brain endothelial cell line subjected either normoxia or to oxygen-glucose deprivation (OGD) and reoxygenation was used to achieve it. Protein expression levels were measured by Western Blot at 24 and 72 h post-treatments, a longer time point analysis than previous works. Here, we determined that the statistically significant ZO-1 level decrease caused by rt-PA at early stages post-treatment (24h) still has not recovered at 72h post OGD. In fact, the decrease is accentuated. Moreover, Claudin-5 seems not to be affected by any treatment at 24h and 72h. We have also demonstrated that OGD significantly increase Cav-1 levels at 72h whereas the administration of rt-PA after OGD does not significantly affect its level.

We can conclude that the changes induced by rt-PA continue altered at long time post OGD (72h) in tight junction proteins. More studies are necessary to determine if rt-PA affects Cav-1 that is strongly increased in OGD conditions.

RECEPTORS I SENYALITZACIÓ CEL.LULAR

P-59. STUDIES OF GPCR LOCALIZATION AND FUNCTIONAL EFFECTS IN BRAIN TISSUE AND IN CULTURED CELLS

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G protein-coupled receptors (GPCRs) constitute the largest family of human membrane proteins and of drug targets. Recent advances in GPCR pharmacology and crystallography have shed new light on signal transduction, allosteric modulation and biased signaling. Over the past decade, an increasing number of studies have reported heteromerization between GPCRs. Many investigations in heterologous systems have provided important indications of potential novel drug design to treat disorders, such as schizophrenia. To explore novel pharmacology of D_2 dopamine (DA) receptor (D_2R) and of metabotropic receptor 5 of glutamate (mGluR5), we are currently investigating the cross-talk between them. Thus, we are determining the possible direct heterodimerization of both receptors, as well as their signaling cross-talk. Preliminary immunohistochemistry (IHC) imaging using anti-mGluR5 antibody show reliable staining in rat brain striatal tissue. These results provide us the basis to explore, by colocalization by IHC, the physical interaction between both receptors. Moreover, since D_2R and mGluR5 have been described as resident in raft microdomains, we decided to detect their presence in rafts isolated from rat, pig and human brain membranes by means of western blot. Results show that rafts extracted with Triton X-100 from several tissues contain monomers of both receptors, as well as putative dimers of mGluR5. To explore some of the signaling possibly modified by receptor cross-talk, we are developing some experimental approaches: determination by HPLC of DA synthesis, detection of G protein activation by ^{35}S-GTPÆ´S binding, or detection of calcium mobilization by microscope imaging using FLUO-4. We observed that the D_2 receptor full agonist Quinpirole inhibits ^{3}H-DA synthesis from ^{3}H-Tyrosine in rat brain striatal slices and also stimulates ^{35}S-GTPÆ´S binding in membranes of CHO-K1 cells transfected with the human D_2 receptor. Moreover, the mGluR5 Ago-PAM VU0360172 increases the calcium mobilization in ST*Hdh* cells from mouse striatum transfected with the human mGluR5.

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P-60. D_1R-GHS-R1A HETEROMER FUNCTIONS PROMOTING COUPLING TO GS-OLF PROTEIN

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Previous studies have suggested that GSH-R1a-mediated signaling depends mostly on Gq coupling, although as also here reported for HEK 293T cells, evidence for Gi-o coupling has also been obtained. Similarly, ghrelin-induced cAMP-PKA signaling has also been reported, but suggested to be independent of Gs-olf proteins. The G protein subtype involved in ghrelin-induced cAMP accumulation in striatal and hippocampal neurons in culture was first investigated by using Gs-olf toxin CTX, Gi-o toxin PTX and the Gq protein inhibitor YM254890. CTX, but not PTX or YM254890 prevented ghrelin-induced cAMP in both preparations, identifying Gs-olf as predominant G proteins coupled to GSH-R1a in neurons. A possible explanation for the unexpected preferential coupling of GHS-R1a to Gs-olf in neurons versus Gi-o in HEK-293T cells could be the presence in neuronal primary cultures of additional receptors that could interact with GHS-R1a or GHS-R1b. D1R is a canonical mediator of adenylyl cyclase activation, particularly in the striatum, and it has been reported to heteromerize with GHS-R1a (Jiang et al., 2006). We then investigated its possible involvement in ghrelin-mediated cAMP accumulation in neurons in culture. In fact, the D_1R antagonist SCH 23390 (1 µM), but not the D_2R antagonist raclopride (1 µM), blocked ghrelin-induced cAMP accumulation in striatal, but not hippocampal, neurons in culture. Consequently, D_1R co-expression can promote a switch in G protein coupling of GHS-R1a, from Gi-o to Gs-olf.

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P-61. TRUNCATED GHRELIN RECEPTOR GHS-R1B MODULATES GHRELIN-INDUCED GHS-R1A SIGNALING

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The truncated non-signaling ghrelin receptor GSH-R1b has been suggested to simply exert a dominant negative role in the trafficking and signaling of the full and functional ghrelin receptor GSH-R1a. Here we reveal a more complex modulatory role of GHS-R1b. Differential co-expression of GHS-R1a and GHS-R1b, both in HEK-293T cells and in striatal and hippocampal neurons in culture, demonstrates that GHS-R1b acts as a dual modulator of GHS-R1a function: low relative GHS-R1b expression potentiates and high relative GHS-R1b expression inhibits GHS-R1a function by facilitating GHS-R1a trafficking to the plasma membrane and by exerting a negative allosteric effect on GHS-R1a signaling, respectively. We found a preferential Gi/o-coupling of the GHS-R1a-GHS-R1b complex in HEK-293T cells and, unexpectedly, a preferential Gs/olf coupling in both striatal and hippocampal neurons in culture.

P-62. AMPAR-TARP STOICHIOMETRY DETERMINES AMPAR BIOPHYSICAL PROPERTIES

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Fast excitatory neurotransmission is mainly carried by AMPA-type glutamate receptors, which are structures formed by 4 receptor pore-forming subunits and several types of auxiliary accessory proteins. Hence, AMPAR biophysical behavior, trafficking properties and consequently AMPAR function reckons basically on these two constituents. Amongst the different auxiliary subunits that stably interact with AMPARs in the central nervous system, transmembrane AMPAR regulatory proteins (TARPs) are the main important and assistant proteins given their high expression and wide distribution through distinct brain areas. They importantly control the trafficking and the gating and pharmacology of AMPARs. For example, TARPs increase single-channel conductance, slow receptor kinetics, accelerate recovery from desensitization or attenuate the natural intracellular block by polyamines of AMPARs. This modulation by TARPs will differentially determine the specific function of a given AMPAR in neurons. Just a few studies have focused attention on the stoichiometry of the AMPAR-TARP complex (number of TARP molecules per AMPAR). In fact, whether a different number of TARPs into the AMPAR complex affect basic intrinsic key features of the receptor have not been addressed. Here we decided to study how the prototypical TARP γ-2 (stargazin) modulates AMPAR behavior depending on different stoichiometries. Thus, by means of electrophysiological recordings in expression systems we have studied AMPAR responses to glutamate using fusion proteins of GluA1-Stargazin to obtain AMPARs with fixed amounts of TARPs. Our results show that most of AMPAR properties are changed differentially depending on the number of TARPs (2 or 4) present into the complex. However, AMPARs need a minimum of 4 TARPs to alter some of their properties, as for example single channel conductance. Given that in the hippocampus some population of neurons display 2 or 4 TARPs depending the area, the variable stoichiometry increase the range of responses in different areas of the brain.

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P-63. MUTATION OF THE PDK1 SUBSTRATE-DOCKING SITE IN THE DEVELOPING BRAIN REDUCES THE POOL OF BASAL AND INTERMEDIATE NEURAL PROGENITORS INDEPENDENTLY OF AKT

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BACKGROUND: The PI3K/AKT signaling pathway plays essential roles during neuronal development. PDK1 activates AKT following the PH-domain mediated colocalization of both kinases to the plasma membrane . Besides AKT, PDK1 also activates twenty-one other members of the AGC family of protein kinases following the binding to a phosphorylated substrate docking site termed PDK1-Interacting Fragment (PIF). Our mouse model, the PDK1$^{fl/fl}$ Nestin-CRE^{+} knock-in mice, express a disrupted form of the PDK1 PIF binding pocket avoiding the activation of all the PI3K cascade except for AKT specifically in brain tissues. The adult PDK1$^{fl/fl}$ CRE^{+} mutant mice cortex show defective axonal interconnectivity, reduced expression of the GABA-synthesizing enzyme GAD67 and layer IV (pyramidal-rich) cell compactation. However, no defects on cell number was found. Behavioural studies indicated that the PDK1$^{fl/fl}$ CRE^{+} mice display a schizophrenia-like phenotype. We aim to study the effects of the PIF-pocket mutation in the developing brain.

RESULTS: PDK1$^{fl/fl}$ CRE^{+} mice exhibited microcephaly starting at embryonic stages, which is strongly accentuated in the adult brains. Specifically, neocortex thickness, but not cortical plate thickness, was reduced in E15,5 PDK1$^{fl/fl}$ CRE^{+} mutant brains. Cell death and proliferation were studied in order to explain this volumetric reduction. PDK1$^{fl/fl}$ CRE^{+} showed reduced proliferation rate in vitro, while cell death was not altered. In vivo analysis also showed that both basal and apical proliferating progenitor pools were reduced. Intermediate progenitors, a pool of still proliferating cells committed with the neuron-linage expansion, were also reduced in number in the PDK1$^{fl/fl}$ CRE^{+} cortex.

DISCUSSION: PDK1$^{fl/fl}$ CRE^{+} microcephaly could be a consequence of a decrease in the proliferating cortical cell number. A cell cycle lengthening or a premature exit of the cell cycle could account for those discrepancies. Remarkably, AKT (which is not affected in our mutant model) is not responsible for these alterations.

P-64. INTERACTIONS BETWEEN GPR55 AND CANNABINOID RECEPTORS

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The endocannabinoid system includes two cannabinoid, CB_1 and CB_2, receptors (CB_1R and CB_2R). Despite evidence pointed to GPR55 as the third cannabinoid receptor, lysophosphatidylinositol appears as the endogenous GPR55 agonist. GPR55 activation triggers intracellular calcium mobilization via Gα13/RhoA, ERK1/2-MAPK activation and cytoskeleton rearrangement. It may mediate some non-CB_1R/CB_2R cannabinoid effects and, accordingly, GPR55 may be activated by cannabinoid compounds: AM251 and SR141716A (rimonabant), are reported as GPR55 agonists, CP55,940 as GPR55 antagonist/partial agonist. It has also been described that GPR55 responds to the endocannabinoids, to delta-9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol in a cell-type and tissue-dependent way.

Using bioluminescence resonance energy transfer (BRET) and in situ proximity ligation assays (PLA) we showed that GPR55 forms heteromers with either CB_1R or CB_2R. The heteromer fingerprints for CB_1-GPR55 and for CB_2-GPR55 have been identified. For CB_1-GPR55 it consists of cross-antagonism (CB_1R antagonists block GPR55 agonist) on ERK1/2 phosphorylation and on GPR55-mediated NFAT activation. CB_2R-GPR55 heteromerization provokes a reduction in GPR55-mediated activation of transcription factors whereas ERK1/2-MAPK activation is potentiated in the presence of CB_2R. CB_2R -mediated signalling is also affected by GPR55. Label-free assays confirmed the cross-talk between the two receptors.More recent and preliminary results show that GPR55 responds to JWH133 and Δ^9-THC. LPI but also cannabinoids are able to trigger intracellular calcium mobilization as detected using a sensor. In transiently transfected cells, Δ^9-THC and LPI increase ß-arrestin recruitment to GPR55, while JWH133 and CP55,940 do not produce any effect. These intriguing results merit further experimental work to better understand cannabinoid-GPR55 receptor interactions.

P-65. THE DISCOVERY OF TWO ANCESTRAL GENE FAMILIES EXPANDS OUR CURRENT REPERTOIRE OF GLUTAMATE RECEPTORS

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Glutamate is the major excitatory neurotransmitter in the central nervous system, acting on ionotropic (iGluRs) and metabotropic (mGluRs) receptors. We have performed a phylogenetic study of the genes coding for these receptors to reconstruct their metazoan evolution. To our surprise this analysis has uncovered a new family of genes coding for ionotropic glutamate receptor subunits and a new family of metabotropic receptors, both lost early in vertebrate evolution. The new family of iGluR subunits appeared prior to the diversification of AMPA, Kainate and Delta type receptors, reason why we have named it A/K/D1. On the other hand, the new family of mGluRs appeared concomitantly with Classes I, II and III and we have thus named it Class IV. We have validated by qPCR the nervous system expression of all genes described in this study in Branchiostoma lanceolatum (Amphioxus). Remarkably, we have demonstrated that amphioxus has more gene families coding for iGluR subunits and mGluRs than vertebrates, seen a greater variety of glutamate receptors in amphioxus. The expression of receptors identified in the amphioxus nerve chord suggests us they are really functional. We conclude that the molecular complexity of the amphioxus is not necessarily lesser than the vertebrates.

P-66. SYNAPTIC ACTIVITY-MODULATED BDNF-TRKB PATHWAY ENHANCES PRESYNAPTIC CPKCβI TO CONTROL NEUROMUSCULAR SYNAPTIC FUNCTION

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One of the benefits of physical activity/exercise on the health of the CNS is to improve the synaptic function (Van Praag-H et al., 1999) and it is well accepted the preponderance of BDNF in mediating these effects (reviewed by Gomez-Pinilla and Hillman, 2013). However, how an increase of synaptic activity-induced muscle contraction can modulate neuromuscular synaptic function through BDNF and its receptor, TrkB, remains unknown. We have recently identified that PKC family is involved in neurotransmitter release when continuous electrical stimulation imposes a moderate activity on the NMJ and that muscle contraction has an important impact on presynaptic PKC isoforms levels, specifically cPKCβI and nPKCε (Besalduch et al., 2010; Obis et al., 2015). Accordingly, the present study hypothesized that muscle contraction is a key regulator of BDNF/TrkB signaling pathway, activating presynaptic cPKC isoforms to modulate synaptic function. ELISA and Western blotting results show that pre- and postsynaptic neuromuscular activity are both responsible for the increase of BDNF levels in skeletal muscle and that nerve induced-muscle contraction regulates TrkB–T1 without affecting TrkB-FL and p75 levels. Moreover, the results show the involvement of BDNF/TrkB signaling pathway induced by pre- and postsynaptic activity on regulation of the presynaptic classical cPKC isoforms (α and βI) and their phosphorylation. We also demonstrate by electrophysiological techniques that cPKCβI is decisively involved in ACh release induced by electrical stimulation. Together, these results provide a mechanistic insight into how synaptic activity-induced muscle contraction could regulate the BDNF/TrkB signalling at the NMJ by enhancing BDNF production and decreasing the TrkB-T1 levels. It further suggests that this signalling pathway could increase presynaptic levels of the cPKCβI isoform to affect neuromuscular neurotransmission.

P-67. ADENOSINE RECEPTORS, MACHRS AND TRKB MODULATE THE DEVELOPMENTAL SYNAPSE ELIMINATION PROCESS AT THE NEUROMUSCULAR JUNCTION

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The development of the nervous system involves an initially exuberant production of neurons that establish excessive synaptic contacts and the subsequent reduction in both neurons and synapses as maturation proceed. Skeletal muscle fibers in the neuromuscular junction (NMJ) become innervated by a single axon at the end of the axonal elimination. The involvement of the muscarinic ACh autoreceptors (mAChR) in the elimination process may allow the direct competitive interaction between nerve endings through a differential activity-dependent ACh release in the synaptic cleft. Here we investigate by quantitative immunohistochemistry the involvement of the individual M_1-, M_2- and M_4-subtypes of mAChRs in the control of the axonal elimination in developing NMJ. We found that in the initial phase of synapse elimination (around P7), the ensemble M_1/M_4 mAChR are involved in delaying axonal loss. However, some days later, at P9, it is fully manifested a constitutive, tonic, muscarinic mechanism, mediated by the subtypes M_1 and M_2, committed to promote axonal disconnection and synapse elimination of the supernumerary nerve terminals in all NMJ during development.

Blocking TrkB receptor pathway (with TrkB-Ig) and the full set of the adenosine receptors (AR, with 8SPT), we found that these receptors similarly to the mAChRs, contribute to hasten axonal elimination. Thus, the three receptor sets promote axonal disconnection at the beginning of the second postnatal week.

P-68. EFFECTS OF OX1R SELECTIVE ANTAGONIST SB-334867 ON CREB EXPRESSION AND ACTIVATION AND BDNF EXPRESSION IN HIPPOCAMPUS AND AMYGDALA, IN SELF-STIMULATED RATS

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Intracranial self-stimulation (ICSS) in the lateral hypothalamus (LH) is a treatment capable of facilitating learning and memory in rats. The Orexin-producing neurons of LH project throughout the brain, including hippocampus and amygdala. Orexin 1 receptors (OX_1R), present in these regions, are implicated in reward behaviours, like ICSS, and it affects neuroplasticity through the activation of long-term plasticity signal pathways. This study aims to determine the effect of ICSS on the activation of memory and plasticity-related proteins including p38 and CAMKII kinases, CREB and BDNF expression levels, and if these effects are altered by the blockade of the OX_1R using its selective antagonist SB-334867.

Four experimental groups (Sham, Sham+SB, ICSS and ICSS+SB) were analysed. The SB-334867 was intraperitoneally injected just before ICSS (or Sham) session. Expression and phosphorylation of CAMKII, p38 and CREB were determined by Western Blot and BDNF quantification by ELISA in hippocampus and amygdala 30 min after treatment. Neither the ICSS treatment nor SB administration modified the activation of p38 or CAMKII in hippocampus and amygdala. No differences of pCREB and CREB levels were observed in hippocampus. However, ICSS significantly decreases amygdalar activation of CREB (Sham > ICSS; P=0.002) and its levels return to basal when both ICSS and SB-334867 were administered (ICSS+SB > ICSS; P=0.007). In addition, SB-334867 decreased total CREB levels (Sham > SB; P=0.032) that reverted when ICSS was applied (ICSS+SB > SB; P=0.001).

Expression of BDNF was significantly increased after ICSS in amygdala (ICSS > Sham; P=0.041) while in hippocampus a similar increase was only observed when SB-334867 was administered (ICSS+SB > Sham+SB; P=0.014). Results reveal an interaction between ICSS and the orexinergic pathway that affects proteins related to synaptic plasticity like CREB and BDNF but neither p38 nor CAMKII kinases in hippocampus and amygdala.

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P-69. NON-CANONICAL FUNCTIONS OF CYCLIND1: EFFECTS ON GABAA RECEPTORS

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Cyclin D1-CDK4 complex has been well characterized as a regulator of G1/S phase transition. In a canonical mechanism, this complex phosphorylates different regulatory substrates in the cell nucleus such as the retinoblastoma protein (RB1), and regulates transcriptional activity. In addition to its nuclear function in cell cycle, Cyclin D1-Cdk4 phosphorylates cytoplasmic substrates involved in the regulation of fibroblasts migration. Also, different data suggests that Cyclin D1 may be involved in neuronal polarization and differentiation: Cyclin D1 has been observed in the cytoplasm during differentiation of neuroblastoma cells and cortical neurons in culture, downregulation of Cyclin D1 in PC12 cells decreases NGF-promoted neurite outgrowth, and Cyclin D1-/- mice show neuronal abnormalities. In our lab Cyclin D1 has been found to interact with the alpha4 subunit of GABAA receptor (GABRA4) by a two-hybrid screen. GABRA4 mediates extrasynaptic inhibition and has been involved in neuronal proliferation, migration and neurite arborization in the adult hippocampal neurogenesis. We have observed that Cyclin D1 and GABRA4 interact in vitro and that CyclinD1-CDK4 complex is able to phosphorylate GABRA4 in a kinase assay. There are 5 putative phosphorylation sites of CDK in GABRA4. We are analyzing the sites of phosphorylation of CyclinD1-CDK in GABRA4 by mass-spectrometry. We aim to study the electrophysiological effects of this phosphorylation and other putative effects on neuritogenesis and migration.

P-70. STUDY ON AUTOPHAGY AND UPR IN PRIMARY AND CHEMORESISTANT GLIOBLASTOMA

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Glioblastoma Multiforme (GBM) is the most common and highly invasive brain tumor, with a poor prognosis and average survival of the patients below 14 months. The standart treatment involves surgery, radiotherapy and chemotherapy with Temozolomide (TMZ), an alquilant agent. Despite of the enforcement of this therapeutic scheme the recurrence of GBM is at that moment inevitable.

Among the mechanisms for tumoral resistance, the study of the endoplasmic reticulum stress (Unfolded Protein Response, UPR) and autophagy as adaptative processes activated in response to nutrient deprivation, chemo and radiotherapy has gained interest in recent years.Here we have investigated the regulation of autophagy and UPR after TMZ treatment in GBM cells. We have generated a GBM cell line resistant to TMZ (A172-R), derived from the A172 cell line. Finally, we have analyzed the autophagy and UPR markers in paired biopsies obtained from patients (primary tumor vs. recurrence of the same patient). Results indicate that TMZ raises the autophagy markers (LC3-II, p62,Beclin, P-AMPK, Atg5 and the dimer Atg5-Atg12), some of them already at 6h after TMZ, and the UPR marker P-eIF4. Similar results were obtained in the chemoresistant cell line. Moreover, resistant cells showed an increased autophagic flux, as revealed by the expression of ptfLC3 construct. Together these findings demonstrate that TMZ is an autophagy inductor. Analysis of the paired biopsies of primary and recurrent GBMs confirmed an increase of the mentioned markers in the recurrences.

On the other hand, we have observed that A172-R overexpresses a T-type isoform of voltage gated calcium channel (Cav3.1) previously associated to a high autophagic flux and which inhibition deregulates autophagy promoting cell death. Ongoing experiments will define if the regulation of Cav3.1 and autophagy in primary and chemoresistant GBM could be a good therapeutic strategy for this tumor.

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P-71. STUDY OF CPT1C FUNCTIONS THROUGH A MUTATED ISOFORM INSENSITIVE TO MALONYL-COA

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Carnitine palmitoyltransferase 1 (CPT1) C is a brain specific isoform of the CPT enzymes family, which is localized in neuronal endoplasmic reticulum. Despite its minimal CPT1 catalytic activity, CPT1C is able to bind malonyl-CoA and long-chain acyl-CoAs. CPT1C KO mice present impaired spatial learning and cognition, motor deficits and deregulation of food intake and energy homeostasis. However, the exact molecular mechanisms mediating these functions and if they depend on malonyl-CoA are still unknown. Therefore, our aim was to evaluate whether CPT1C function in neurons is regulated by malonyl-CoA. To carry out this study we constructed the mutant CPT1CM589S by site directed mutagenesis to alter its binding site to malonyl-CoA. Methionine 589 had been previously described to be crucial for malonyl-CoA binding to CPT1A. We studied the involvement of malonyl-CoA in the interaction of CPT1C with the subunit 1 of glutamate AMPA receptors (GluA1), a recently well-known interaction, and in the regulation of GluA1 expression levels. For this purpose, CPT1C and CPT1CM589S were overexpressed in cortical neurons and immunoprecipitation and western blot assays were performed. We found that CPT1CM589S maintained the interaction with GluA1 and increased GluA1 protein levels similarly to CPT1C overexpression. These results suggest that the CPT1C role in GluA1 receptor expression regulation is malonyl-CoA-independent, and hence confirming the functionality of CPT1CM589S. In conclusion, we have developed a tool to study whether CPT1C is acting as a malonyl-CoA sensor in neurons and how this interaction further regulates its binding with other neuronal proteins.

P-72. EARLY ASTROCYTE AND NEURON DYSFUNCTION DUE TO LIPID DYSHOMEOSTASIS

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X-linked adrenoleucodistrophy (X-ALD) is a neurometabolic disorder caused by mutations in the *abcd* genes, which lead to the loss of function of the ALD protein (ALDP), an ATP-binding cassette responsible for the import of very long-chain fatty acids (VLCFA) into peroxisomes to their further degradation by β-oxidation. The impairment of ALDP results in the accumulation of VLCFA leading to progressive demyelination within the central (CNS) and peripheral nervous system (PNS) and impaired steroidogenesis in the adrenal cortex. However, the cellular compartmentalization in CNS and how they relate to function remains unclear. We aim to evaluate these defects by modelling the adult form of the disease in neuron and astrocyte cultures from rat cortex and hippocampus. Cells had the *abcd1* and *abcd2* genes silenced, and were treated with 50 µM C26:0 using α-cyclodextrin as a carrier. Results obtained indicate that VLCFA causes alteration of calcium responses, endoplasmatic reticulum stress and mitochondrial β-oxidation dysfunction in astrocytes, spine disassembly in neurons and a time-dependent slight neuronal and astrocytic death. These data suggest that astrocyte and neuronal dysfunction, and hence circuit activities, precedes demyelination and death. Thus, early brain cell dysfunction emerges as a possible therapeutic target in pre-symptomatic patients to arrest the progression of the disease.

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P-73. NEUROBLASTOMA CELLS (SH-SY5Y) CULTURE SIGNALING RESPONSE TO STIMULATION WITH EPO: NEUROPROTECTION AND INOSITOL PATHWAYS

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The study of cell signaling triggered by neuroprotective cytokines is reaching high interest in neurodegenerative diseases. The hormone erythropoietin (EPO) has a well-described role in the homeostatic response against hypoxia. In addition to erythroid progenitors, EPO receptor has been found in neurons and preliminary studies indicate that it plays a role in neuroprotection against brain hypoxia.

The neuroprotective action of the neuronal EPO receptor would be conducted by triggering PLC/InsP3 and PI3K/AKT signaling pathways. It has been described that an increase in cellular calcium level could play an excitotoxic role in neurons, and, for instance, this fact could cause cell death. N the other hand, the inactivation of the proapoptotic factor FoxO3A by the PI3K/AKT system has been associated with neuroprotection.

The main purpose of this study is to explore these possibilities using SH-SY5Y cultured cells that express EPO receptor. Confluent cultures were stimulated with EPO and cells were extracted at different times. In the resulting lysates was evaluated total and phosphorylated FOXO3A, PI3K, AKT and PLC (Western Blot); cAMP, ATP, ADP, GTP and soluble phosphorylated inositols (HPLC analysis).

The results indicate that EPO stimulation acts on the levels of 1,4-$InsP_2$ isomer and specifically in the third minute on the 1,3,4-$InsP_3$ isomer. These preliminary results suggest an implication of phosphoinositide molecules in neuronal EPO signaling. It was also observed an increase in the PI3K and Akt phosphorylation after 5 minutes of stimulation. However, the Foxo3A phosphorylation remained constant during the whole stimulation. These results suggest that Akt could contribute to the inactivation of other proapoptotic factors, like Bad.

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COGNICIÓ, COMPORTAMENT I TRASTORNS MENTALS

P-74. MOLECULAR AND BEHAVIORAL ALTERATIONS INDUCED BY MATERNAL ALCOHOL BINGE CONSUMPTION IN MICE

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Maternal alcohol consumption has long-term adverse consequences since it occurs during key periods of the brain development. In utero alcohol exposure leads to a range of long-lasting morphological and behavioral deficiencies known as fetal alcohol spectrum disorders, associated with a higher risk of later developing drug abuse. In this study, we sought to test whether alcohol binge drinking during prenatal period promotes long-term behavioral and molecular effects in the offspring. Pregnant C57BL/6J female mice underwent a procedure that models binge alcohol drinking (drinking-in-the-dark test) during either the gestation period, or during both the gestation and the lactation period. Then, adult male offspring were assessed for their cognitive function (object recognition test and Y-maze tasks). Recognition memory was not significantly affected by early alcohol exposure (EAE), but executive functioning was impaired in mice exposed to alcohol during prenatal and lactation periods. In addition, increased levels of neuroinflammatory markers (TLR4, NF-kappaB/p65, IL-1β) were found in the prefrontal cortex (PFC) and hippocampus (HPC) of EAE mice. Interestingly, our results indicate that EAE leads to a deficit in myelination since the expression of structural myelin proteins (MAG, MBP, PLP) was diminished. Finally, H3K9, H4K5 and H4K12 acetylation increased in the PFC of EAE mice, indicating a long-lasting epigenetic modification of chromatin. Altogether, our results reveal that maternal binge alcohol consumption induces cognitive function impairments on the adult offspring and leads to long-term molecular alterations in the brain.

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P-75. NEONATAL FINASTERIDE ADMINISTRATION DECREASES DOPAMINE RELEASE IN NUCLEUS ACCUMBENS AFTER ALCOHOL AND FOOD PRESENTATION IN ADULT MALE RATS

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Endogenous concentrations of the neurosteroid (NS) allopregnanolone (AlloP) during neonatal stages are crucial for the correct development of the central nervous system. Manipulations of the neonatal AlloP levels have been related with alterations of certain cerebral structures as well as with altered adolescent and adult behaviours. Some of these alterations include neuronal mechanisms (e.g. dopaminergic pathways) and behavioural traits (e.g. anxiety and novelty-directed locomotion) that could be related to vulnerability to drug abuse. In a recent work we reported that the neonatal administration of AlloP or finasteride (Finas), an inhibitor of the enzyme 5α-reductase needed for AlloP synthesis, altered the voluntary consumption of ethanol and the ventrostriatal dopamine (DA) levels in adulthood, suggesting that neonatal NS manipulations can increase alcohol abuse vulnerability in adulthood. Thus, the aim of the present work is to analyse if manipulations of neonatal AlloP alter the dopaminergic response in the nucleus accumbens (NAcc) during alcohol intake in rats. We administered AlloP or Finas from postnatal day (PND) 5 to PND9. At PND98, we measured alcohol consumption using a two-bottle free-choice model (ethanol 10% (v/v)+glucose 3% (w/v), and glucose 3% (w/v)) for 12 days. On the last day of consumption, we measured the DA and 3,4-dihydroxyphenylacetic acid (DOPAC) release in NAcc in response to ethanol intake. The samples were obtained by means of in vivo microdialysis in freely moving rats, and the DA and DOPAC levels were determined by means of high-performance liquid chromatography analysis (HPLC). The results revealed that neonatal Finas increased ethanol consumption during several days of the consumption phase, and decreased the DA release in the NAcc in response to solutions (ethanol+glucose) and food presentation. Taken together, these results emphasise the importance of neonatal NS levels on neurodevelopment, suggesting for the first time that neonatal NS alterations can affect adult alcohol rewarding properties.

PSI2012-36646

P-76. NEW BEHAVIORAL INSIGHTS ON GENE – TOXIC INTERACTIONS: THE ROLE OF APOLIPOPROTEIN E AND CHLORPYRIFOS IN MODULATING ATTENTION, MOTIVATION AND INHIBITORY CONTROL

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More and more evidence endorses the contribution of organophosphate pesticides –and chlorpyrifos (CPF) in particular- in boosting neurobehavioral disorders. Most experimental research focuses on learning and memory processes, while other cognitive domains remain understudied. The isoforms of the human apolipoprotein E (apoE) confer on their carriers innate differences in cognitive skills, but data on this topic are still limited. Likewise, apoE isoforms have been proven to differently influence the brain neuromodulatory system. We aimed at assessing whether *APOE* genotypic variability differently modulate the effects of CPF on attentional performance, inhibitory control, and motivation. Human apoE targeted replacement adult female mice (apoE2, apoE3, and apoE4) were trained to stably perform the 5-choice serial reaction time task prior being fed CPF (3.75 mg/kg body weight/day) for 4 consecutive weeks. After the exposure, a CPF-free period was established to assess recovery. All individuals acquired the task, but apoE2 mice were the most gifted learners. ApoE4 mice displayed increased basal premature and perseverative responding, condition that was reversed after the exposure to CPF. Overall, the pesticide induced protracted impairments in sustained attention and motivation, and it reduced anticipatory responding. ApoE3 mice exhibited delayed attentional disruptions throughout the wash-out period. Taken together, these findings attest potential links between *APOE4* genotype and the cholinergic system, and provide notable evidence on the emergence of CPF-related attentional and motivational deficits.

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P-77. LONG TERM EFFECTS OF SEX, GENOTYPE AND POSTNATAL EXPOSURE TO PESTICIDES ON RECOGNITION MEMORY

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Pesticides are extensively used worldwide in agriculture and in residential applications for pest control. A massive use of pesticides may cause adverse effects in non-target organisms, representing a risk to public health. One of the most widely used pesticides is chlorpyrifos (CPF), belonging to the organophosphate family. Several studies have related its exposure with neurobehavioral disorders, being of special interest the effects observed in child population. CPF exerts its toxicity by inhibiting acetylcholinesterase enzyme. Human apolipoprotein E (apoE) is an important cholesterol carrier involved in lipid homeostasis. The apoE4 isoform has been identified as a susceptibility factor for cognitive impairment, higher cardiovascular risk and Alzheimer disease in humans. In the present study we investigated the long term neurobehavioral effects of a postnatal exposure to the pesticide CPF in human apoE4 targeted replacement mice, as well as in wild C57BL/6 mice. Mice were exposed to 0 or 1mg/kg/day of CPF during the postnatal period 10-15. Associative recognition memory was assessed 45 days after by means of an object recognition test, a two-trial cognitive paradigm based on the innate tendency of rodents to explore a novel object in preference to a familiar one. In order to study the different contribution of the acetylcholine and GABA neurotransmitters systems, pharmacological challenges were carried out during the test. The selected drugs were scopolamine, rivastigmine and alprazolam. Results from behavioral procedures suggest genotype and sex affected exploration pattern, being females the ones expressing higher activity. Moreover, lower levels of exploration were observed in apoE4 mice. In basal conditions there were no significant differences between groups, although the pharmacological challenges revealed differences between groups suggesting complex interactions between sex, genotype and postnatal CPF exposure. Overall, our results highlight the importance of using pharmacological challenges to unmask subtle or latent defects.

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P-78. LACK OF NEURONAL IL-6 PROMOTES ACTIVITY AND REDUCES ANXIETY BUT HAS NO EFFECT ON SPATIAL LEARNING IN MICE

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Interleukin-6 (IL-6) is a pleitropic cytokine involved in both inflammatory and non-inflammatory responses. Regarding the latter, several studies in total knock-out mice (IL6-KO) have demonstrated a role in the control of body weight, body temperature, stress response, emotional reactivity, synaptic plasticity, learning, sleep and nervous system development. The role in several behavioral parameters is controversial, different groups have found conflicting results with different paradigms. Given the different cellular sources of IL-6, both in the periphery and in the central nervous system, which could be confounding the results, we propose a conditional knock-out approach to elucidate the role of each source in different aspects of behavior. Here we present initial results with the neuronal knockout (neu-IL6KO). We have evaluated 8-11-week-old male and female neu-IL6KO mice and their floxed controls in the open field (OF), hole board (HB), elevated plus maze (EPM) and Morris water maze (MWM) tests. Our results show that neu-IL6KO mice perform more rearings and have more activity, both in external and internal areas, in the OF and HB tests. In the EPM, a test more focused on anxiety, neu-IL6KO mice also have more activity and rearings, do more head dippings and spend more time in open arms than floxed controls, suggesting less anxiety. Regarding hippocampal-related learning, as assessed with the Morris water maze test, no differences were seen in either spatial or reversal learning. These results show different roles of neuronal IL-6 on normal brain physiology, some of which go in different directions than IL6-KO or astrocyte IL6-KO mice.

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P-79. BEHAVIOURAL CHARACTERIZATION OF MICROGLIAL IL-6 KO MICE

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Interleukin-6 (IL-6) is a pleiotropic cytokine that controls the immune system and influences the central nervous system (CNS) in normal and pathological conditions. Neurons, glia and other CNS cells synthesize and respond to IL-6. Several studies using total knock-out mice (IL6-KO) showed that IL-6 takes part in a number of physiological functions such as the stress response, emotional reactivity, synaptic plasticity, learning and nervous system development.Given the cell-specific production of IL-6 in the CNS, they might be participating in different functions in the regulation of behaviour. Our group previously reported that astrocytic IL-6 is involved to various degrees in the control of locomotor activity, anxiety and exploratory behaviours as well as survival and adult body weight. Here we present initial results of phenotypical characterization using a battery of behavioral tests (Open Field, Hole-Board and Elevated Plus Maze) with the microglial knock-out (mic-IL6 KO), generated with the Cre-lox technology, and floxed mice as a control.

Ministerio de Economía y Competitividad.

P-80. NEONATAL NEUROSTEROIDS LEVELS ALTERATION ON ALCOHOL ABUSE VULNERABILITY: PARTICIPATION OF SEROTONIN 5HT3 RECEPTORS

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Neonatal neurosteroids such as allopregnanolone (AlloP) are crucial for brain development and adult behaviour. AlloP alterations during the first two weeks of life modify novel environments exploration, anxiety and pre-attentional behaviours, and increases ventrostriatal serotonin levels in adulthood. Given that, neonatal AlloP levels alteration could lead to a pattern of novelty/sensation-seeking in adulthood, and it has been reported that this pattern may be related to an increased vulnerability to drug use and abuse. In order to test a possible role of serotonin systems, we have tested whether the effects of a 5-HT3 serotonin receptor antagonist (ondansetron) on alcohol intake is affected by neonatal AlloP levels alteration. We injected AlloP, finasteride (an AlloP synthesis inhibitor) or vehicle from postnatal day 5 to 9. In adulthood (PN70), behavioural tests of novelty/sensation-seeking were performed. Afterwards, it was carried out a process of voluntary alcohol intake with access to two bottles (alcohol 10%+glucose 3% versus glucose 3%) one hour daily for two weeks. Ondansetron (0.01mg/kg, 0.1mg/kg or vehicle) was injected in the initial (days 1, 2 and 3) and the end (days 11, 12 and 13) of the consumption phase. Results indicated that the animals administered with AlloP showed an increased exploratory behaviour and presented the lowest alcohol consumption. Instead, finasteride-treated subjects showed an increased novelty object recognition together with greater alcohol consumption than the rest of experimental groups. Interestingly, ondansetron (0,1mg/kg) decreased alcohol consumption only in finasteride-treated animals. In conclusion, manipulation of neurosteroids levels such as AlloP in crucial stages of development seems to play an important role in the vulnerability to drug abuse, suggesting a possible implication of the serotonin 5HT3 receptors.

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P-81. CANNABIDIOL ATTENUATES COCAINE-INDUCED PLACE PREFERENCE AND REINSTATEMENT OF COCAINE SELF-ADMINISTRATION BEHAVIOR

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The administration of cannabinoid derivatives as a therapeutical approach has raised promising evidence to treat neuropsychiatric disorders, including drug addiction (Devinsky et al., 2014; Prud et al., 2015). Cocaine addiction remains an untreated substance use disorder with undeniable deleterious long-term consequences (Volkow, 2010). Here, we used conditioned place preference (CPP) and self-administration (SA) paradigms to investigate whether cannabidiol (CBD), the main non-psychoactive constituent of cannabis plant, would affect the behavioral neuroadaptations supporting the reinforcing properties of cocaine administration in male CD-1 mice. For the CPP experiments, CD-1 mice were pretreated during 10 days with CBD 5, 10, 20 and 30 mg/kg, by ip route. 15 days later, saline- and CBD-pretreated animals (n ≈ 17/group) were subjected to an unbiased CPP procedure. For the cocaine intravenous (i.v.) SA experiments, a different set of animals (male CD-1) were pretreated during 10 days with CBD 20 mg/kg, ip. Acquisition, extinction and reinstatement of cocaine self-administration behavior were evaluated (Soria et al., 2008).

Our results show that CBD 10 and 20 mg/kg impaired the development of cocaine-induced CPP. This effect was not observed at lower (5 mg/kg) and higher (30 mg/kg) doses of CBD, suggesting an inverted U-shaped pharmacological profile of the CBD effects on cocaine-induced CPP. Regarding SA experiments, CBD 20 mg/kg did not alter the acquisition of the task, the total intake of cocaine, extinction behavior, or compulsive intake under a progressive ratio schedule of cocaine administration. Instead, CBD attenuated the cocaine-seeking behavior reinstated by exposure to a priming dose of cocaine (10 mg/kg).

Taken together, our results suggest that CBD interferes with brain reward mechanisms responsible for the expression of the acute reinforcing properties of cocaine and its ability to reinstate drug-seeking behavior in relapsing conditions, thus postulating CBD as a feasible therapeutic strategy to still working with in cocaine addiction research.

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P-82. MOUSE MODEL OF ASTROGLIAL GLUTAMATE TRANSPORTERS KNOCKDOWN IN INFRALIMBIC CORTEX INDUCES A DEPRESSIVE PHENOTYPE

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Emerging evidences suggest that dysregulations of glutamatergic neurotransmission in prefrontal cortex (PFC) are involved in the pathophysiology of depression. Astrocytes regulate excitatory neurotransmission by removing synaptic glutamate via GLAST and GLT-1 transporters. We hypothesized that reduced expression of GLAST and GLT-1 in mouse infralimbic-PFC would induce depressive-like behaviors due to disabled glutamate reuptake. We microinjected small interfering RNAs (siRNA) targeting GLAST or GLT-1unilaterally into prelimbic (PrL) or infralimbic (IL) cortices of mice and examined cellular and behavioral effects. Local GLAST-siRNA microinfusion in IL (4.2 nmol) reduced selectively GLAST mRNA and protein levels to ~80% of control mice 24h post-administration. GLAST knockdown mice exhibited depressive-like behaviors including anhedonia and, increased immobility time in the TST (125% of controls) and FST (128% of controls). Likewise, intra-IL GLT-1-siRNA infusion (4.2 nmol) reduced GLT-1 expression (~70% of controls) and mice showed a depressive-like phenotype in TST, FST and sucrose preference. Depressive-like IL symptoms were reverted by citalopram (10 mg/kg, i.p.). Conversely, intra-Prl microinfusion of both siRNAs did not affect behavioral responses despite of the reductions of GLAST and GLT-1-1 expression. Downregulation of GLAST and GLT-1 remained diminished for at least 3 day and recovered by the 7th day after acute infusion. Despite local veratridine infusion in the dorsal raphe nucleus (DRN) of control and knockdown mice evoked similar 5-HT release, reduced basal 5-HT levels in DRN were detected in IL GLAST-siRNA or GLT-1-siRNA treated mice. Local perfusion of GABAA receptor antagonist bicuculline (30 and 100µM) increased DRN-5-HT release. This effect was more marked in knockdown than control mice, suggesting higher activation of GABA interneurons in siRNA-treated mice. Overall, these findings improve our understanding of the pathophysiology of depression, suggesting differences in connectivity between IL and PrL cortices with other cortical and subcortical regions and helping to identify novel targets in antidepressant drug development.

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P-83. UNRAVELLING THE CONTRIBUTION OF ADENOSINE A2A RECEPTOR TO THE NEUROBIOLOGY OF SCHIZOPHRENIA

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Schizophrenia is a chronic and severe neuropsychiatric disorder associated with a neurodevelopmental origin. Its pharmacotherapy mostly relies on restoring a dysregulated striatal dopamine and prefrontal cortex glutamate neurotransmission. Importantly, as the current treatment is often insufficient to coat all the symptomatology (i.e. negative and cognitive symptoms) the search for alternative and/or complementary neurotransmitter systems involved in the aetiology of schizophrenia constitutes a big contest in psychiatry these days. Indeed, adenosine, a well-known neuromodulator in the central nervous system, has been highlighted because its relationship with both dopaminergic and glutamatergic neurotransmission.

Here we studied the relationship between adenosine A2A receptors (A2ARs) and dopamine D2 receptor (D2R) expression in a pharmacological animal model of schizophrenia, namely chronic phencyclidine (PCP) treatment. Interestingly, while PCP treatment induced psychotic-like symptoms in mice it also promoted a significant and differential increase of A2AR and D2R expression in the striatum. Next, as it has been demonstrated that the genetic blockade of A2AR induced cognitive impairments and anatomical changes related to psychotic symptoms in mice1 we aimed to analyse the expression of D2R in the striatum of KO-A2AR mice. Remarkably, the D2R levels in the knockout (KO) animal were significantly increased when compared to the wild type (WT) littermates. Finally, in an attempt to manipulate the D2R content in the KO-A2AR mice, and to correlate with the psychotic-like symptoms described for, we assessed the electroconvulsive therapy (ECT). Thus, while ECT increased striatal D2R expression in WT animals it promoted a significant reduction in the KO-A2AR mice. Importantly, this ECT-mediated D2R reduction in the KO was followed with a concomitant improvement in the KO-A2AR mice's sensorimotor gating impairment as measured by the pre-pulse inhibition (PPI) test. Overall, a good correlation between striatal D2R expression and psychotic-like symptoms could be stablished and the A2AR content might be a contributing factor to.

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P-84. *CX3CR1-V249I* VARIANT MODIFY DISEASE PROGRESSION IN HISTOPATHOLOGICAL CONFIRMED LATE ONSET ALZHEIMER'S DISEASE

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Neuroinflammation and microglial dysfunction have a prominent role in the pathogenesis of late-onset Alzheimer's disease (LOAD). *CX3CR1* is a microglia-specific gene involved in microglia-neuron crosstalk. It turned out as a genetic modifier in ALS, but no studies have been performed to investigate its influence on LOAD's outcome. We designed an open, pragmatic, case-control retrospective study including a total of 475 subjects (205 pathologically confirmed AD cases and 270 controls). We analyzed the association of the CX3CR1- V249I functional variants (rs3732379) with survival time, progression rate of neurofibrillary pathology according to Braak' staging system, age at onset (AAO) and risk of suffering LOAD. We used five inheritance models in the association analysis. We found that individuals heterozygous for *CX3CR1*-V249I, under an over-dominant genetic model, presented a lower neurofibrillary pathology progression (OR= 0.42, 95%CI [0.23, 0.74], p=0.003, adj-p=0.015) than the other genotypes. These results provide further evidence of the involvement of CX3CR1 and microglia/macrophages in the pathogenesis of LOAD.

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P-85. ANALYSIS OF PERIPHERAL BLOOD BIOMARKERS OF BRAIN RESILIENCE IN VETERAN RUGBY PLAYERS

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Physical exercise practiced regularly is known to improve general health and to reduce the risk of age-related cognitive loss and Alzheimer's disease. Furthermore, there are some evidences of a cognitive improvement in physically active young to middle-aged adults. We hypothesized that middle age men engaged in a sport team as veteran practitioners have built brain resilience that can be detected with the analysis of peripheral blood markers. We recruited healthy veteran rugby players (n=24) and controls with low physical activity (n=25) aged 45-65 years and devoid of serious concussions or brain trauma. All subjects were submitted to neuropsychological testing that showed that the player's group had improvement in some immediate learning and memory mechanisms. Notably, the cognitive benefice of sport practice is not new, but rugby players had not been previously analyzed. Next we tested the expression of selected genes related to neurotrophism, inflammatory and redox homeostasis and the SIRT family of longevity genes in total blood by qPCR. Analyses were also performed in blood samples of a group of young people with low physical activity (n=21) aged 15-25 years, that was added for comparison. Interestingly, middle aged subjects showed lower levels of expression of SIRT1 and the ratio SIRT1:SIRT2 (proposed as neuroprotective sirtuin ratio), than the group of youths, whereas the sport group showed intermediate values. Furthermore, SIRT1 and SIRT1:SIRT2 correlated with diverse neuropsychological parameters of cognitive response. Also, the cytokine IL10 and the ratio IL10:IL6 (predictive of positive anti-inflammatory outcome), correlated with several parameters of moderate physical activity. Finally, the expression of BDNF correlated with hours of physical exercise and especially with intense activity. In conclusion, the changes of gene expression induced by physical exercise in peripheral blood cells positively correlated with physiological and cognitive conditions indicative of brain resilience in the analyzed cohorts of middle aged men.

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X SIMPOSI DE NEUROBIOLOGIA

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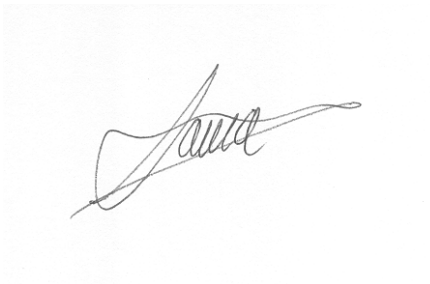
Barcelona, 7th of October 2016



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