with the suport of:

Amb la col·laboració del Vicerectorat
de Recerca, Innovació i Transferència

Coordinació Institucional del CSIC a Catalunya

Societat Catalana de BIOLOGIA

IX Simposi de Neurobiologia Experimental

22 i 23 d'octubre de 2014

Institut d'Estudis Catalans

Barcelona

Scientific and organizing committee:

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GENERAL PROGRAM

Day 1 (Wednesday, Oct 22th 2014)		Day 2 (Thursday, Oct 23th 2014)	
8:30-9:15	Registration		
9:15-9:30	**Welcome and Meeting Presentation**		
9:30-11:00	Session 1A **GLIAL CELLS AND NEUROINFLAMMATION** Room: Prat de la Riba Session 1B **NEURONAL DEATH, NEUROPROTECION, NEUROREGENERATION** Room: Pere Coromines	9:30-11:00	Session 3A **NEUROPSYCHIATRIC DISORDERS** Room: Prat de la Riba Session 3B **SIGNALING IN THE CNS** Room: Pere Coromines
11:00-12:00	Coffee-break and pòsters 1	11:00-12:00	Coffee-break and pòsters 2
12:00-13:00	PLENARY LECTURE1: **Hugh Perry** (University of Southampton) **NEUROINFLAMMATION: INTERACTIONS BETWEEN LOCAL AND SYSTEMIC INFLAMMATION IN THE BRAIN** Room: Prat de la Riba	12:00-13:00	**2nd "Ramon Turró" Award** ACCEPTANCE LECTURE **Albert Adell** (IIBB, CSIC, Barcelona) **ESTRÈS I DEPRESSIÓ: PAPER DELS TRANSMISSORS CEREBRALS** Room: Prat de la Riba
13:00-15:00	Lunch	13:00-15:00	Lunch
15:00-16:30	Session 2A **OTHER DISORDERS OF THE NERVOUS SYSTEM** Room: Prat de la Riba Session 2B **DEVELOPMENT, NEUROGENESIS AND STEM CELLS** Room: Nicolau d'Olwer	15:00-16:30	Session 4A **NEURODEGENERATIVE DISORDERS** Room: Prat de la Riba Session 4B **NNEUROTRANSMISSION** Room: Nicolau d'Olwer
16.30-16.45	Short break	16:30-17:15	Coffee-break and pòsters 2
16.45-17.45	PLENARY LECTURE2: **Rafael Fernandez Chacón** (Universidad de Sevilla-IBIS) **PRESYNAPTIC DYSFUNCTION AND NEURODEGENERATION IN THE ABSENCE OF A SYNAPTIC VESICLE CO-CHAPERONE: WHAT HAPPENS BEYOND THE NERVE TERMINALS?** Room: Prat de la Riba	17.15-18.15	PLENARY LECTURE3: **Isabelle Mansuy** (ETH, Zurich) **NON-GENOMIC INHERITANCE OF THE EFFECTS OF TRAUMA IN EARLY LIFE THROUGH SPERM NON-CODING RNAS IN MICE** Room: Prat de la Riba
17.45-18-30	Beer & Posters 1	18.15-18.30	**Best poster Award** **Closing**

DETAILED PROGRAM

WEDNESDAY 22nd OF OCTOBER

Room	
8.30	Registration IEC
9.15-9.30	**Welcome and Meeting Presentation**
9.30-11.00	
Prat de la Riba	**SESSION 1A (O1-O6)** **GLIAL CELLS AND NEUROINFLAMMATION** Chairman: Hugh Perry (University of Southampton) Luis Pardo (INc, UAB) Marta Pulido-Salgado (UB, IDIBAPS) Isabel Pérez de Puig (IIBB, CSIC, IDIBAPS) Elena Galea (INc, UAB) Francesc Miró (IIBB, CSIC, IDIBAPS) Ester Bonfill (IIBB, CSIC, IDIBAPS)
Pere Coromines	**SESSION 1B (O7-O12)** **NEURONAL DEATH, NEUROPROTECION, NEUROREGENERATION** Chairman: Pere Boadas (UdG) Joan Romaní (UB) Gerard Esteban (INc, UAB) Helena Masanas (IBEC) Ariadna Arbat (UAB) Pascual Torres (IRB Lleida) Xavier Roa (UIC)
11.00-12.00	Coffee-break and posters (1-35)
12.00-13.00	
Prat de la Riba	PLENARY LECTURE 1: **NEUROINFLAMMATION: INTERACTIONS BETWEEN LOCAL AND SYSTEMIC INFLAMMATION IN THE BRAIN** **Hugh V Perry** University of Southampton, UK Room: Prat de la Riba
13.00-15.00	Lunch
15.00-16.30	
Prat de la Riba	**SESSION 2A (O13-O18)** **OTHER DISORDERS OF THE NERVOUS SYSTEM** Chairman: Jordi Llorens (UB, IDIBELL) Elena I Cazacu (U Lleida) Ambika Periyakaruppiah (U Lleida) Macarena Pozo (UIC) Ping Sun (Inc, UAB) Silvia Fuentes (UAB) Joan Ramon Margalef (URV)
Nicolau d'Olwer	**SESSION 2B (O19-O23)** **DEVELOPMENT, NEUROGENESIS AND STEM CELLS** Chairman: Marta Pascual (UB) Marta Morey (UB) Alejandra Fernández (UB) Marco Straccia (UB, IDIBAPS) Núria Suelves (UB) Ramón Martínez (UB)

16.30-16.45 Short break

16.45-17.45
Prat de la Riba

PLENARY LECTURE 2
PRESYNAPTIC DYSFUNCTION AND NEURODEGENERATION IN THE ABSENCE OF A SYNAPTIC VESICLE CO-CHAPERONE: WHAT HAPPENS BEYOND THE NERVE TERMINALS?

Rafael Fernández-Chacón
Universidad de Sevilla

17.45-18.30 Beer & Posters (1-35)

THURSDAY 23th OF OCTOBER

9.30-11.00
Prat de la Riba

SESSION 3A (O24-O29)
NEUROPSYCHIATRIC DISORDERS
Chairman: Anna Castanyé (CSIC, IDIBAPS)

Helena Palma (UB, CIBERSAM)
Claudia Prats (UB, CIBERSAM)
Jordi Soler (UB)
Nerea Abasolo (IISPV, URV, CIBERSAM)
Alexander Stojanovic-Pérez (IISPV, URV, CIBERSAM)
Alba Valiente (IISPV, URV, CIBERSAM)

Pere Coromines

SESSION 3B (O30-35)
SIGNALING IN THE CNS
Chairman: Silvia Ginés (UB, IDIBAPS)

Andrés Martín-Quiros (IBEC)
Maria Casas (UB)
Abel Eraso (INc, UAB)
Lluís Cordón (INc, UAB)
Laura Nadal (URV)
Silvia Pittolo (IBEC)

11.00-12.00 Coffee-break and posters (36-71)

12.00-13.00
Prat de la Riba

Ceremony for the 2nd "Ramon Turró" Award to the most cited Neuroscience article 25 years after its publication

2nd Ramon Turró ACCEPTANCE LECTURE
ESTRÈS I DEPRESSIÓ: PAPER DELS TRANSMISSORS CEREBRALS
Albert Adell
IIBB, CSIC-IDIBAPS, Barcelona
Room: Prat de la Riba

13.00-15.00 Lunch

15.00-16.30
Prat de la Riba

SESSION 4A (O36-O41)
NEURODEGENERATIVE DISORDERS
Chairman: Jordi Bové (IRVH)

Sara Fernandez (UB, IDIBAPS)
Cristina Alvarez-Zaldiernas (UB)
Rafael Alcalá (UB, IDIBAPS)
Núria Martín Flores (UB)
Arnaldo Parra-Damas (INc, UAB, CIBERNED)
Phil Sanders (UB, IDIBAPS)

Nicolau d'Olwer	**SESSION 4B (O42-47)** **NEUROTRANSMISSION** Chairman: Jordi Ortiz (INC, UAB) Gemma Navarro (UB) Francisco J Lopez-Murcia (IDIBELL) Mercè Izquierdo-Serra (IBEC) Esther Gratacòs-Batlle (UB, IDIBELL) Rut Fadó (UIC) Xavier Altafaj (IDIBELL)
16.30-17.15	Coffee-break and posters (36-71)
17.15-18.15 Prat de la Riba	PLENARY LECTURE 3: **NON-GENOMIC INHERITANCE OF THE EFFECTS OF TRAUMA IN EARLY LIFE THROUGH SPERM NON-CODING RNAS IN MICE** **Isabelle Mansuy** ETH, Zürich
18.15-18.30	Best poster award Evaluation Committee: Carles Saura (coordinator, UAB) Lilian Enríquez (UAB) Francesc Miró (CSIC, IDIBAPS) Marco Straccia (UB, IDIBAPS) Xavier Xifró (UdG)
18.30	Closing

PLENARY LECTURES

PLENARY LECTURE 1:

NEUROINFLAMMATION: INTERACTIONS BETWEEN LOCAL AND SYSTEMIC INFLAMMATION IN THE BRAIN

Hugh Perry

University of Southampton

During progression of a number of neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, and prion diseases there is an innate immune response in the brain. This innate immune response is characterised by an increase in the density of the microglia, and their 'activation' as judged by alterations in their morphology and the upregulation or de novo synthesis of macrophage antigens. We have studied the innate immune response in prion disease, an animal model of chronic neurodegeneration. As the disease progresses there is evidence of microglia activation and their phenotype is dominated by an anti-inflammatory profile. Proliferation and priming of the microglia is driven by colony-stimulating-factor-1 (CSF1) and interleukin-34 (IL-34) binding to the CSFR1. Systemic inflammation has a profound impact on the phenotype of these microglia that appear to be 'primed' by the ongoing neurodegeneration. Systemic inflammation leads to exaggerated pro-inflammatory cytokine synthesis in the brain, relative to naive animals, exaggerated sickness behaviour and acceleration of components of disease progression. Understanding how systemic co-morbidities contribute to progression of chronic neurodegenerative disease offers a route to slowing disease progression and improving the quality of life of those with neurodegenerative disease.

PLENARY LECTURE 2:

PRESYNAPTIC DYSFUNCTION AND NEURODEGENERATION IN THE ABSENCE OF A SYNAPTIC VESICLE CO-CHAPERONE: WHAT HAPPENS BEYOND THE NERVE TERMINALS?

Rafael Fernandez Chacón

Instituto de Biomedicina de Sevilla (IBiS, Hosp.Univ. Virgen del Rocío/CSIC/Universidad de Sevilla), Dpto. de Fisiología Médica y Biofísica and CIBERNED, Sevilla, Spain

Nerve terminals are able to maintain the continuous release of neurotransmitters during extended periods of time at locations far away from the cell soma. For example, presynaptic terminals from tonic motorneurons receive from 300.000 to 500.000 action potentials per day (Hennig and Lomo, *Nature* 1985) imposing on SNARE complexes involved in membrane fusion a heavy-duty cycling of protein folding and unfolding reactions. Cysteine String Protein-alpha (CSP-alpha) is a synaptic vesicle protein that, together with Hsc-70 and SGT (small glutamine-rich protein), forms a chaperone complex essential to maintain a functional pool of the SNARE protein SNAP25 and to promote SNARE complex assembly (Chandra et al., Cell 2005; Sharma et al. *Nat. Cell Biol.* 2011). Interestingly knock-out mice lacking CSP-alpha suffer from early lethality due to presynaptic degeneration (Fernández-Chacón et.al., *Neuron* 2004). We have recently found that motorneurons require CSP-alpha to maintain the readily releasable vesicular pool and synaptic vesicle recycling (Rozas., et al., *Neuron* 2012). Interestingly, in central neurons, we have shown that CSP-alpha prevents activity-dependent degeneration of GABAergic synapses in high firing rate parvalbumin-positive neurons, indicating that high-neural activity increases synapse vulnerability and CSP-alpha is essential to maintain presynaptic function under a physiologically high-activity regime (García-Junco-Clemente et al., *JNeurosci.* 2010). In my talk I will discuss recent unexpected findings that uncover a deregulation of adult neurogenesis at the dentate gyrus and a surprising strong demyelination of central axons in the brain of CSP-alpha KO mice. We think that knock-out mice lacking CSP-alpha offer a particularly useful model to investigate how neural circuits react to progressive presynaptic dysfunction and neurodegeneration. In addition, our results might reveal unanticipated functions of CSP-alpha beyond the maintenance of synaptic vesicle trafficking at the nerve terminals.

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PLENARY LECTURE 3:

NON-GENOMIC INHERITANCE OF THE EFFECTS OF TRAUMA IN EARLY LIFE THROUGH SPERM NON-CODING RNAS IN MICE

Isabelle Mansuy

Brain Research Institute, University/ETH Zürich, Winterthurerstrasse 190, Zürich, Switzerland.
Email: mansuy@hifo.uzh.ch

The etiology and expression of behaviors in mammals are strongly influenced by environmental factors, whether positive or negative. Such factors are particularly critical during early postnatal life and can exert their influence across life. When positive and favorable, they can facilitate the proper development and expression of behavioral responses, but when traumatic and stressful, they can alter behavior and lead to cognitive disorders and psychopathological symptoms. In particular, traumatic events in early life are strong risk factors for conditions such as borderline personality disorder, bipolar depression and antisocial behaviors. Further, while such disorders can strongly affect the individuals directly exposed to trauma, they are also often inherited by the offspring and similarly affect behavioral responses in first and second generations. The biological mechanisms responsible for the transmission of stress-induced symptoms from parent to offspring remain poorly understood but have been postulated to involve epigenetic mechanisms. This talk will present an experimental model of chronic traumatic stress in early life in mice that provides initial evidence for the implication of non-genomic mechanisms in the expression and inheritance of the effects of early trauma on behavior. This mouse model shows that chronic and unpredictable maternal separation combined with unpredictable maternal stress during the first two weeks of postnatal life results in multiple pathological symptoms in the offspring. It induces cognitive deficits, altered social behaviors and social interactions, and depressive-like symptoms in adult individuals. At the same time, such traumatic experience also leads to stress resilience and favors behavioral flexibility in some conditions. These symptoms are pronounced and persist throughout life. Further strikingly, they are transmitted to the following offspring across two generations, through both females and males. At a molecular level, the behavioral symptoms are associated with epigenetic alterations involving different targets, including components of the HPA pathway, ion channels, neurotransmitter receptors, and mitochondrial regulators. There is evidence that these molecular alterations correlate with persistent changes in DNA methylation at the promoter-associated CpG island in several genes, and that these changes are present both, in the brain of the offspring and the germline of their father. Further to DNA methylation, other epigenetic mechanisms involving regulation by non-coding RNAs and possibly histone posttranslational modifications are also involved. These findings suggest that epigenetic processes largely contribute to the impact of negative environmental factors in early life on adult behavior, and its inheritance.

ESTRÈS I DEPRESSIÓ: PAPER DELS TRANSMISSORS CEREBRALS

Albert Adell
Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC)

Les situacions d'estrès produeixen una sèrie de canvis fisiològics i de comportament, la finalitat dels quals és mantenir la integritat biològica de l'organisme davant una estimulació aversiva. Les modificacions de la utilització de determinats neurotransmissors en el sistema nerviós central formen part de les principals conseqüències de l'exposició a l'estrès. Aquest augment de la utilització comporta, a la vegada, un augment de la síntesi de transmissors, que té com a finalitat, mantenir l'homeòstasi. Quan l'exposició a l'estrès és prolongada, pot produir una depleció de la concentració d'aquests neurotransmissors, principalment les monoamines com serotonina, dopamina i noradrenalina, que no pot ser compensada per la corresponent síntesi. És en aquests casos quan es poden desenvolupar patologies associades com és el cas de la depressió. Això no obstant, els canvis en la transmissió monoaminèrgica no és l'única causa de la depressió. Ni tan sols són actors aïllats, donat la forta interacció que hi ha entre aquests i d'altres transmissors. Avui en dia s'accepta que la característica més rellevant dels estats depressius és l'alteració del bon funcionament i bona comunicació entre circuits formats per diferents àrees cerebrals i implicats en l'emoció, cognició, atenció, etc. La missió dels antidepressius, doncs, serà restablir un bon funcionament d'aquests circuits, tot recuperant les concentracions idònies d'aquestes monoamines i, a la vegada, tornar a obtenir una adequada resposta a l'estrès.

ORAL COMUNICATIONS

Session 1A. Glial Cells and Neuroinflammation

O-1. Luis Pardo. THE TARGETED ACTIVATION OF CREB IN ASTROCYTES IS NEUROPROTECTIVE IN BRAIN INJURY BY A DUAL AND OPPOSITE REGULATION OF ENERGY METABOLISM AND INFLAMMATION

O-2. Marta Pulido-Salgado. MICROGLIAL C/EBPβ DEFICIENT MICE: CHARACTERIZATION OF THE MODEL AND NEUROPROTECTION IN EAE

O-3. Isabel Pérez de Puig. NEUTROPHILS IN MOUSE AND HUMAN ISCHEMIC STROKE

O-4. Elena Galea. A DOMAIN-BASED TOPOLOGICAL ANALYSIS IN APP/PS1 TRANSGENIC MICE REVEALS THAT ASTROCYTES DO NOT MIGRATE TO AMYLOID-β PLAQUES

O-5. Francesc Miró-Mur. DYNAMYCS OF PROINFLAMMATORY Ly6Chi MONOCYTES WITH IMMUNOSUPRRESSIVE FEATURES AFTER BRAIN ISCHEMIA IN MICE

O-6. Ester Bonfill-Teixidor. IL-4 EXPRESSION AFTER STROKE AND ALTERNATIVE MICROGLIA/MACROPHAGE ACTIVATION

Session 1B. Neuronal death, Neuroprotecion, Neuroregeneration

O-7. Joan Romaní. PARKIN LOSS OF FUNCTION CONTRIBUTES TO RTP801 ELEVATION AND NEURODEGENERATION IN PARKINSON'S DISEASE

O-8. Gerard Esteban. MULTIFUNCTIONAL METAL-CHELATING, CHOLINESTERASE AND MONOAMINE OXIDASE INHIBITORS FOR THE POTENTIAL TREATMENT IN ALZHEIMER'S DISEASE.

O-9. Helena Masanas. USE OF XENOPUS TROPICALIS TO INVESTIGATE THE REWIRING OF THE NERVOUS SYSTEM

O-10. Ariadna Arbat. ACTIVITY DEPENDENT THERAPIES MODULATE THE SPINAL CHANGES THAT MOTONEURONS SUFFER AFTER A PERIPHERAL NERVE INJURY

O-11. Pascual Torres. EFECTES DE LA SUPLEMENTACIÓ DIETÈTICA AMB ÀCID DOCOSAHEXAENOIC EN EL MODEL MURÍ D'ESCLEROSI LATERAL AMIOTRÒFICA G93A-hSOD1

O-12. Xavier Roa. CPT1C IS EXPRESSED IN MESENCHYMAL STEM CELLS AND PROMOTES CELL SURVIVAL UNDER METABOLIC STRESS INDUCED BY GLUCOSE DEPRIVATION OR 2-DEOXYGLUCOSE

Session 2A. Nervous system disorders: others

O-13. Helena Irina Cazacu. EFECTE DE LES TINTES DELS TATUATGES SOBRE EL SISTEMA NERVIÓS PERIFÈRIC

O-14. Ambika Periyakaruppiah. SURVIVAL MOTOR NEURON (SMN) PROTEIN LEVEL IS REGULATED BY AUTOPHAGY MODULATORS IN MOTONEURONS FROM A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY (SMA)

O-15. Macarena Pozo. HYPOTHALAMIC CPT1C IS INVOLVED IN NUTRIENT PARTITIONING IN LIVER AND MUSCLE DURING FASTING

O-16. Ping Sun. INVOLVEMENT OF SSAO/VAP-1 IN OXYGEN-GLUCOSE DEPRIVATION-MEDIATED DAMAGE USING THE ENDOTHELIAL HSSAO/VAP-1-EXPRESSING CELLS AS EXPERIMENTAL MODEL OF CEREBRAL ISCHEMIA

O-17. Silvia Fuentes. EFECTOS SEXO-DIMÓRFICOS DEL ESTRÉS TEMPRANO EN LA ATRIBUCIÓN DE SALIENCIA INCENTIVA EN LA ETAPA ADULTA EN RATAS

O-18. Joan Ramon Margalef. ESTUDI DE LA FISIOPATOLOGIA DE LA PRODUCCIO DE PUNTS GALLET MIOFASCIALS EN RATOLINS

Session 2B. Development, neurogenesis and stem cells

O-19. Marta Morey. DECODING WIRING SPECIFICITY IN THE FLY VISUAL SYSTEM.

O-20. Alejandra Fernández. IDENTIFICATION OF GENES INVOLVED IN PHOTORECEPTOR WIRING SPECIFICITY

O-21. Marco Straccia. HIGH-THROUGHPUT GENE EXPRESSION ANALYSIS OF HUMAN STRIATAL NUCLEI DEVELOPMENT IN CONTROL AND HUNTINGTON'S DISEASE PATIENTS

O-22. Núria Suelves. PAPER DE P75NTR DURANT EL NEURODESENVOLUPAMENT EN LA MALALTIA DE HUNTINGTON

O-23. Ramón Martínez-Mármol. ROLE OF MEMBRANE MICRODOMAINS IN NETRIN-1-DEPENDENT GROWTH CONE CHEMOREPULSION

O-1

THE TARGETED ACTIVATION OF CREB IN ASTROCYTES IS NEUROPROTECTIVE IN BRAIN INJURY BY A DUAL AND OPPOSITE REGULATION OF ENERGY METABOLISM AND INFLAMMATION

Luis Pardo[1,2], Agatha Schlüter[3,4], Angel Barco[5], Mercè Giralt[1,6], Juan M. Hidalgo[1,6], Roger Mateu[1,2], Manuel Portero Otín[7], Lydia Giménez-Llort[1,8], Roser Masgrau[1,2], Aurora Pujol[3,4,9] and Elena Galea[1,2,9]

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[9]Institució Catalana de Recerca i Estudis Avançats (ICREA)

The transcription factor CREB is protective in injury and neurodegeneration, but the role of neuronal vs astrocytic CREB is unknown. We have generated a mouse with targeted over-expression of a constitutively active CREB (VP16-CREB) in astrocytes by crossing TetO-VP16 and TtA-GFAP mice. VP16-CREB/GFAP mice show expression of VP16 in astrocytes -as detected by immunohistochemistry – primarily in cerebellum, hippocampus and outer layers of cortex. We determined the role of astrocytic CREB in the outcome of brain injury by subjecting wild type and VP16-CREB/GFAP mice to a focal cryolesion in the frontal cortex. At 3 days post-lesion, WT mice presented a necrotic region filled with macrophages surrounded by a rim of gliotic tissue, while transgenic mice showed reduced macrophage infiltration and increased gliosis. A DNA array and subsequent enrichment analysis of the damaged zone revealed alterations caused by the cryolesion and the transgene in 127 KEGG pathways. A more restricted analysis ($p<0.0001$) showed 27 altered pathways in the cryolesion of which 16 were also regulated by VP16-CREB, corresponding to 5 major functions: energy metabolism, inflammation, structural reorganization, genomic stability and stress response. DNA array results, together with qPCR validation and immunohystochemical analysis showed that VP16-CREB increased the expression of metabolic enzymes while it decreased the expression of extracellular matrix components and reduced inflammatory processes and neuronal death. We posit that glial VP16-CREB protects the brain against bioenergetic failure following injury and secondary damage due to macrophage infiltration, while rendering the tissue more conducive to neurite regeneration by reducing extracellular matrix components.

O-2

MICROGLIAL C/EBPβ DEFICIENT MICE: CHARACTERIZATION OF THE MODEL AND NEUROPROTECTION IN EAE

Pulido-Salgado M[1], Serratosa J[2], Valente T[1,2], Castillo P[3], Matalonga J[4], Straccia M[1,2], Solà C[2], Saura J[1]

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We have recently shown that the b-zip transcription factor C/EBPβ regulates proinflammatory gene expression in microglia. This suggests that microglial C/EBPβ inhibition could have therapeutic potential in CNS disorders with a pathogenic neuroinflammatory component. To test this hypothesis in animal models of neurodegenerative diseases we have generated mice with specific microglial C/EBPβ deficiency. Mice with Cre expression under the microglial/macrophage promoter LysM were crossed with C/EBPβ fl/fl mice and double mutants LysMCre-C/EBPβ fl/fl were selected. These animals showed normal fertility, survival and histology in contrast to mice with full C/EBPβ deficiency. In primary microglial cultures of LysMCre-C/EBPβ fl/fl mice, lack of C/EBPβ was observed in virtually 100% of microglial cells, whereas astrocytes showed normal C/EBPβ expression. Microglial C/EBPβ absence resulted in the almost total blockade of NO production induced by LPS+IFNγ and in altered bacterial phagocytic function. Acute isolation of adult mice microglia revealed a high proportion (90%) of C/EBPβ negative microglial cells also in vivo. Finally, because C/EBPβ was markedly upregulated by experimental autoimmune encephalomyelitis (EAE) in wild-type mice, control and LysMCre-C/EBPβ fl/fl mice were subjected to EAE. LysMCre-C/EBPβ fl/fl mice presented delayed onset and markedly attenuated EAE severity. Altogether, these findings support the hypothesis that C/EBPβ is a key regulator of proinflammatory gene expression in microglial cells and that its inhibition has therapeutic potential. Supported by La Marató de TV3 (110530) and Instituto de Salud Carlos III, Spain-FEDER funds, European Union (PI10/378 and PI12/00709).

O-3

NEUTROPHILS IN MOUSE AND HUMAN ISCHEMIC STROKE

Isabel Perez-de-Puig, PhD[1&], Francesc Miró, PhD[2&]; Maura Ferrer-Ferrer, Ms[1,2] ; Ellen Gelpi MD,PhD[3], Jordi Pedragosa BS[1], Carles Justicia, PhD[1,2], Xabier Urra MD, PhD[2,4], Angel Chamorro, MD, PhD[2,4], Anna M. Planas, PhD[1,2]*

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Neutrophils are rapidly recruited to the sites of infection as part of the innate immune response against invading pathogens. However, neutrophils also respond to sterile inflammation and release of their toxic cargo can damage the tissues. Stroke triggers a strong inflammatory reaction but the location and role of neutrophils in the ischemic brain is still a matter of controversy. Here we investigated neutrophil trafficking to the brain in murine models of permanent middle cerebral artery occlusion and fatal cases of human ischemic stroke. Flow cytometry analyses of mouse brain cells revealed progressive neutrophil accumulation peaking at 24 hours. Confocal microscopy showed neutrophils trapped in capillaries and adhered to post-capillary venules. In addition, during ischemia neutrophils progressively left the circulation at the leptomeninges, reached the surface of the ischemic cortex and the Virchow-Robin spaces of perforating cortical vessels where they accumulated. After 24 hours of sustained ischemia, perivascular neutrophils gained access to the cortical parenchyma across the basement membranes. At this time, some neutrophils presented signs of NET formation by showing citrullination of histone-3, chromatin decondensation, and/or projection of DNA and histones out of the cells. Neutrophil extravasation at the leptomeninges, accumulation in perivascular spaces, and presence in the parenchyma was confirmed in postmortem human stroke brain tissue. We concluded that the leptomeninges are gateways for neutrophil access to perivascular spaces of cortical brain vessels and that long-lasting ischemia is needed for neutrophil activation compromising the integrity of the basement membranes and allowing their infiltration to the cortical brain parenchyma.

O-4

A DOMAIN-BASED TOPOLOGICAL ANALYSIS IN APP/PS1 TRANSGENIC MICE REVEALS THAT ASTROCYTES DO NOT MIGRATE TO AMYLOID-ß PLAQUES

Elena Galea[1,2], Will Morrison[3], Eloise Hudry[4], Michal Arbel-Ornath[4], Brian J. Bacskai[4], Teresa Gómez-Isla[4], H. Eugene Stanley[3], Bradley T. Hyman[4]

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The observation that GFAP immunopositive astrocytes cluster around plaques in Alzheimer's disease leads to the widespread assumption that amyloid-ß plaques attract astrocytes, but an alternate explanation is that stationary astrocytes near plaques become selectively GFAP immunopositive, and therefore give the appearance of migrating towards plaques. Recent studies indeed show that astrocytes do not move in injury. Here we re-examine whether astrocytes migrate to plaques by analyzing with mathematical functions and computer modeling the topology of astrocytes, and alterations thereof by plaques, in 3D images obtained by 2-photon microscopy from APP/PS1 transgenic mice. We found that, in normal mice, cortical astrocyte topology fits a model akin to a liquid of hard spheres that exclude each other in a confined space. Plaques do not disturb this arrangement at large distances but, locally, they cause subtle outward shifts of the astrocytes located in three tiers around plaques. These data are consistent with a model in which astrocytes respond to plaques by changing phenotype and over-expressing GFAP, rather than changing position. This evidence may help to redefine the role of astrocytes in Alzheimer´s disease.

O-5

DYNAMYCS OF PROINFLAMMATORY Ly6Chi MONOCYTES WITH IMMUNOSUPRRESSIVE FEATURES AFTER BRAIN ISCHEMIA IN MICE

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We investigated the responses of different monocyte subsets to stroke in a model of permanent middle cerebral artery occlusion in mice. Using strategies of adoptive transfer of reporter monocytes and monocyte depletion we show that stroke promotes the maturation of $Ly6C^{hi}$ monocytes to non-classic $Ly6C^{lo}CD43^{hi}CCR2-$ monocytes and stimulates the release of immature $Ly6C^{hi}$ from body reservoirs to the blood. $Ly6C^{hi}$ monocytes secreted cytokines after activation, showed high phagocytic activity, and were able to inhibit T cell proliferation, showing that they had proinflammatory but also direct immunosuppressive properties. The $Ly6C^{hi}CD43^{lo}CCR2+$ cells were the predominant monocytes recruited to the ischemic tissue where they acquired features of macrophages over time. Acute pharmacologic CCR2 blockade reduced infarct volume showing that proinflammatory $Ly6C^{hi}$ monocyte infiltration in the acute phase of stroke is detrimental. However, stroke also increased $Ly6C^{hi}$ monocyte numbers in the spleen where the observed immunosuppressive features suggest they can control T cell reactivity.

O-6

IL-4 EXPRESSION AFTER STROKE AND ALTERNATIVE MICROGLIA/MACROPHAGE ACTIVATION

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Stroke triggers inflammation that exacerbates brain tissue damage. The inflammatory reaction is naturally set up to clear the necrotic tissue and comprises a complex dynamic process evolving through various steps from initiation to resolution which are not fully elucidated. IL-4 is a cytokine mainly produced by lymphocytes that promotes an alternative anti-inflammatory M2 phenotype in macrophages. Mice deficient in IL-4 show worse stroke outcome suggesting that IL-4 may play a role in brain ischemia. The aim of this study was to identify the IL-4 expression in the brain after ischemia and investigate the effects of IL-4 on the inflammatory response of glial cells.

Permanent middle cerebral artery occlusion (pMCAO) in mice produces low levels of IL-4 mRNA expression from 6 hours to 4 days post-ischemia, but it strongly increased at day 7. At this time, the expression of several inflammatory markers was attenuated while that of molecules involved in alternative M2 phenotype and tissue repair increased. Treatment of cultures of murine glia with recombinant IL-4 increasingly upregulated the expression of the M2 markers such as arginase-1 up to 48h while M1 markers are inhibited. This profile in glia was dependent on Jak1/Jak3/Stat6 pathway and requires a new protein synthesis. Microglia treated with IL-4 showed a reduced pro-inflammatory response when challenged with LPS.

We suggest that initial proinflammatory milieu set after brain ischemia is followed by the up-regulation of IL-4 and of markers of alternative M2 phenotype and that JAK/STAT pathways are involved. M1 glia can be reprogrammed by IL-4 switching to a M2 phenotype.

O-7

PARKIN LOSS OF FUNCTION CONTRIBUTES TO RTP801 ELEVATION AND NEURODEGENERATION IN PARKINSON'S DISEASE

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Mutations in the *PARK2* gene are associated with an Autosomal Recessive form of Juvenile Parkinsonism (AR-JP). These mutations affect parkin solubility and impair its E3 ligase activity leading to a toxic accumulation of proteins within susceptible neurons that results in a slow but progressive neuronal degeneration and cell death. Here, we report that RTP801/REDD1, a pro-apoptotic negative regulator of survival kinases mTOR and Akt, is one of such parkin substrates. We observed that parkin knockdown elevated RTP801 in sympathetic neurons and neuronal PC12 cells while ectopic parkin enhanced RTP801 poly-ubiquitination and proteasomal degradation. In parkin knockout mouse brains and in human fibroblasts from AR-JP patients with parkin mutations, RTP801 levels were elevated. Moreover, in human postmortem PD brains with mutated parkin, nigral neurons were highly positive for RTP801. Further consistent with the idea that RTP801 is a substrate for parkin, the two endogenous proteins interacted in reciprocal co-immunoprecipitates of cell lysates. A potential physiological role for parkin-mediated RTP801 degradation is indicated by observations that parkin protects neuronal cells from death caused by RTP801 over-expression by mediating its degradation, while parkin knockdown exacerbates such death. Similarly, parkin knockdown enhanced RTP801 induction in neuronal cells exposed to the Parkinson's disease mimetic 6-OHDA and increased sensitivity to this toxin. This response to parkin loss-of-function appeared to be mediated by RTP801 since it was abolished by RTP801 knockdown. Taken together these results indicate that RTP801 is a novel parkin substrate that may contribute to neurodegeneration caused by loss of parkin expression or activity.

O-8

MULTIFUNCTIONAL METAL-CHELATING, CHOLINESTERASE AND MONOAMINE OXIDASE INHIBITORS FOR THE POTENTIAL TREATMENT IN ALZHEIMER'S DISEASE.

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Alzheimer's Disease (AD) is an age-related neurodegenerative process characterized by progressive memory loss and other cognitive impairments. Although its etiology has not yet been elucidated, some AD hallmarks including β-amyloid (Aβ) deposits, Tau protein hyperphosphorylation, oxidative stress or deficits of acetylcholine (ACh) have been described to play a key role in both pathophysiology and progression of the disease. The current pharmacological AD therapy mainly comprises the use of acetylcholinesterase inhibitors (AChEI) and other single-target therapies with limited success. Consequently, a new pharmacological strategy based on the use of Multi-Target-Directed Ligands (MTDL) has recently emerged as an appropriate therapeutic approach to addressing the multifaceted nature of AD. Thus, in this context, we have developed novel multifunctional molecules as hybrids of Donepezil+Propargylamine+8-Hydroxyquinoline (DPH) moieties able to simultaneously inhibit both cholinesterases and monoamine oxidases with metal-chelating, anti-Aβ and antioxidant properties as a potential enhancement for the current pharmacological therapy of AD. From all derivatives tested, differing on the length of the carbon chain linker and the presence of a ciano moiety, DPH4 and DPH6 displayed the most interesting profile as dual ChE/MAO inhibitors with potent Cu(II)/Fe(II)-chelating activities. In addition, potent antioxidant properties were revealed with both molecules by different *in vitro* methods (ORAC-FL, DPPH and LP). DPH4 also exhibited *in vitro* moderate inhibition on both self-mediated and AChE-mediated $A\beta_{1-42}$ aggregation. Toxicity assays showed DPH6 to induce less toxicity than Donepezil at high concentrations in Hep295 cells *in vitro*. Finally, passive avoidance task on mice with experimentally induced amnesia exhibited that DPH6 significantly decreased scopolamine-induced learning deficits in healthy adult mice.

O-9

USE OF XENOPUS TROPICALIS TO INVESTIGATE THE REWIRING OF THE NERVOUS SYSTEM

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We are using *Xenopus* tadpoles to gain a better understanding on the mechanisms controlling rewiring of the nervous system upon injury. To this aim we established a Xenopus colony and developed transgenesis procedures to obtain genetically modified animals. Olfactory circuitry is well described in Xenopus tadpoles and offers a suitable platform to study reformation of synaptic connections. Three main cellular populations exist in the olfactory bulb: periglomerular, mitral and granular neurons. In the present work we investigated: i) the time course of olfactory nerve reconnectionreformation after injury and ii) the changes in neuronal populations during reformation of pre-existing circuitry. Both objectives were addressed by cutting a single olfactory nerve in tubb-2-GFP tadpoles, which express EGFP under neural-beta-tubulin promoter. We observed that the mean time required for nerve reformation is 4 days although standard nerve thickness is never re-achieved. Regarding neuronal densities we did not observe a significant change neither in mitral ($1{,}8\times10^{-3}$ cells/μm^3 in the cut bulb and $1{,}9\times10^{-3}$ cells/μm^3 in the control bulb; time = 6 days after cut) nor in granular cells ($6{,}3\times10^{-4}$ cells/μm^3 in the cut bulb and $7{,}3\times10^{-4}$ cells/μm^3 in the control bulb; time = 9 days after cut). However, periglomerular cells showed a 3-fold increase in density for damaged olfactory bulbs (6×10^{-4} cells/μm^3 in the cut bulb and $1{,}8\times10^{-4}$ cells/μm^3 in the control bulb; time=6 days after cut). These data evidence the large regenerative capabilities of the Xenopus olfactory system and provides a new experimental approach to investigate some of the cellular and synaptic changes occurring during rewiring of neuronal circuits.

O-10

ACTIVITY DEPENDENT THERAPIES MODULATE THE SPINAL CHANGES THAT MOTONEURONS SUFFER AFTER A PERIPHERAL NERVE INJURY

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Injury of a peripheral nerve leads to target denervation, but also induces massive stripping of spinal synapses on axotomized motoneurons, with disruption of spinal circuits. Even when regeneration is successful, unspecific reinnervation and the limited reconnection of the spinal circuits impair functional recovery. The aim of this study was to describe the changes that axotomized motoneurons suffer after peripheral nerve injury and how activity-dependent therapies and neurotrophic factors can modulate these events. We observed a marked decrease in glutamatergic synapses, with a maximum peak at two weeks post-axotomy, which was only partially reversed with time. This decrease was accompanied by an increase in gephyrin immunoreactivity and a disintegration of perineuronal nets (PNN) surrounding the motoneurons. Direct application of neurotrophins at the proximal stump was not able to reverse these effects. In contrast, activity-dependent treatment, in the form of treadmill running, reduced the observed destructuring of perineuronal nets and the loss of glutamatergic synapses two weeks after injury. These changes were proportional to the intensity of the exercise protocol. Blockade of sensory inputs from the homolateral hindlimb also reduced PNN immunoreactivity around intact motoneurons, and in that case treadmill running did not reverse that loss, suggesting that the effects of exercise on motoneuron PNN depend on increased sensory activity. Preservation of motoneuron PNN and reduction of synaptic stripping by exercise could facilitate the maintenance of the spinal circuitry and benefit functional recovery after peripheral nerve injury.

O-11

EFECTES DE LA SUPLEMENTACIÓ DIETÈTICA AMB ÀCID DOCOSAHEXAENOIC EN EL MODEL MURÍ D'ESCLEROSI LATERAL AMIOTRÒFICA G93A-hSOD1

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L'esclerosi lateral amiotròfica (ELA) és la malaltia de la motoneurona amb més prevalença en humans adults, essent més freqüent en homes, amb una proporció 3:1 respecte les dones. Aquesta malaltia està caracteritzada per una pèrdua progressiva de motoneurones superiors i inferiors que condueix a paràlisi, atròfia muscular i mort al cap de 3-5 anys.
Encara que les causes de la degeneració de motoneurones en l'ELA es desconeixen, l'àcid docosahexaenoic (DHA, *Docosahexaenoic acid*), un dels àcids grassos més importants en l'homeòstasi del sistema nerviós, es veu reduït en medul·la espinal de pacients d'ELA.
En aquest estudi es mostra, després d'una suplementació dietètica amb DHA, l'efecte gènere-específic del DHA augmentant la supervivència dels mascles però no de les femelles en el model de ratolí transgènic G93A-hSOD1 (model d'ELA familiar). Aquesta intervenció provoca un augment dels nivells de DHA en medul·la espinal i indueix canvis en altres àcids grassos involucrats en la inflamació. La suplementació amb DHA disminueix la carbonilació de proteïnes, el marcador 8-oxodG i la resposta de dany al DNA, posant de manifest el paper del DHA en regular l'estrès oxidatiu en proteïnes i DNA, a més, provoca un augment en els nivells de sintaxina-3, és a dir: una major disponibilitat de proteïnes involucrades en les sinapsis.
En conclusió, es demostra la funció neuroprotectora del DHA en la medul·la espinal en un model experimental murí d'ELA, suggerint un benefici de la suplementació dietètica amb DHA en pacients d'ELA potencialment major en homes que en dones.

O-12

CPT1C IS EXPRESSED IN MESENCHYMAL STEM CELLS AND PROMOTES CELL SURVIVAL UNDER METABOLIC STRESS INDUCED BY GLUCOSE DEPRIVATION OR 2-DEOXYGLUCOSE

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Carnitine palmitoyltransferase 1C (CPT1C) belongs to CPT1 enzymes family which control fatty acid entrance to the lumen of mitochondria for its oxidation. Nevertheless, the CPT1C isoform, which is expressed mainly in the brain, has a very low catalytic activity and it is localized in the endoplasmic reticulum. Our research group has demonstrated that, at biochemical level, CPT1C controls ceramide synthesis in neurons and, at physiological level, CPT1C regulates peripheral energy metabolism, spatial learning and motor function. Recently, another research group has demonstrated that CPT1C is also expressed in tumor cells, conferring them the ability to survive in hypoglycemic and hypoxia situations. In the present work, we have studied whether CPT1C is expressed in human adult stem cells because, like neuronal or tumor cells, they remain in the organism the whole life of it. To deal with this issue, we obtained mesenchymal stem cells (MSC) from human dental pulp of the third molar and analyzed the CPT1C expression. We found that CPT1C was expressed in human MSC at higher level than in human brain.

Moreover, we submitted hMSC to different types of metabolic stress and analyzed whether CPT1C was a protector factor. We found that CPT1C over-expression increased survival of hMSC against cellular damage induced by glucose depletion or 2-deoxyglucose. Interestingly, when glucose was deprived, endogenous CPT1C expression also increased.

Nowadays, we are interested in the molecular pathway by which CPT1C exerts its protective effects. Furthermore, we want to elucidate whether CPT1C also promotes survival in mouse cortical neuronal primary cultures in response to metabolic stress.

O-13

EFECTE DE LES TINTES DELS TATUATGES SOBRE EL SISTEMA NERVIÓS PERIFÈRIC

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INTRODUCCIÓ: La composició de les tintes dels tatuatges, la seva farmacocinètica en el cos humà i els riscos potencials de la inoculació de les tintes són desconeguts. Així quan un anestesista troba tatuatges a la zona lumbar on pretén realitzar una punció lumbar o administrar una anestèsia epidural no sap com procedir: hi ha risc d'arrossegar colorants i que aquests siguin neurotòxics? Davant el dubte alguns opten per seccionar la pell i suturar després del procediment en qüestió.
MATERIAL I MÈTODES: S'han utilitzat nervis ciàtics de rata Sprague-Dawley. S'han fet registres de la conducció nerviosa on s'han testat diferents concentracions dels compostos majoritaris de les tintes així com tintes comercials (Intenze i Millenium). Les incubacions han estat de 60-90minutos. S'han avaluat les amplituds dels potencials d'acció compostos (*peack to peack*).
RESULTATS: Hem obtingut un bloqueig de la conducció nerviosa amb la majoria de les tintes comercials testades (~ 80% de bloqueig, P <0.05). Els components majoritaris de les tintes no son tant tòxics com les tintes mateixes i tenen accions molt tardanes o no en tenen cap. Els registres control realitzats mostren una oscil·lació màxima de 20% al final dels 90 minuts.
CONCLUSIONS: Els colorants que s'usen habitualment en els tatuatges blocan la conducció nerviosa. Els compostos químics que donen color a les tintes tenen poca acció sobre la conducció nerviosa. Per tant la acció detectada als colorats es fruit de la sinergia entre compostos químics.

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O-14

SURVIVAL MOTOR NEURON (SMN) PROTEIN LEVEL IS REGULATED BY AUTOPHAGY MODULATORS IN MOTONEURONS FROM A MOUSE MODEL OF SPINAL MUSCULAR ATROPHY (SMA)

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Spinal muscular atrophy is a neuromuscular disorder caused by the reduction of survival motor neuron (SMN) protein resulting in muscle weakness and atrophy. SMN protein function is the assembly of small nuclear ribonucleoprotein and pre-mRNA splicing requirements. In vitro studies of spinal cord motoneurons reveal that SMN protein reduction causes neurite degeneration and cell death. The main objective of our study is to identify molecular and cellular mechanisms involved in this motoneuron degeneration in order to analyze possible therapeutic targets. Autophagy is a basic cellular degradation pathway of long lived proteins or organelles through the action of lysosome engulfment. It is unclear that accumulation of autophagosome is preventive or degenerative. In the present work we studied the autophagy marker LC3-II in motoneurons. We show changes of LC3-II in SMN-reduced motoneurons obtained from in vivo and in vitro models. Treatment with the proteasome inhibitor MG132 increases both SMN protein level and LC3-II in cultured motoneurons. Our experimental data indicate an increase of the autophagosome accumulation in SMN-reduced motoneurons and the role of the proteasome in the regulation of SMN and autophagy in these cells.

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O-15

HYPOTHALAMIC CPT1C IS INVOLVED IN NUTRIENT PARTITIONING IN LIVER AND MUSCLE DURING FASTING

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Maintenance of energy homeostasis is coordinated by the crosstalk between peripheral organs and the central nervous system; more specifically, mediobasal hypothalamus (MBH) plays a key role in the control of energy balance and glucose homeostasis. In previous studies we have demonstrated that CPT1C, the brain-specific isoform of carnitine palmitoyltransferase 1, is involved in both leptin and ghrelin signalling in MBH. Moreover, other authors have evidenced that CPT1C KO mice present a hypometabolic phenotype both during fasting and in high fat diet, showing higher sensibility to obesity and insulin resistance. Therefore, the main aim of this work was to elucidate which metabolic pathways in the peripheral tissues are modulated by hypothalamic CPT1C.

Here we demonstrate that CPT1C KO mice have a defective fasting response due to an impaired switch in fuel utilization by peripheral tissues. CPT1C KO mice are unable to induce the adequate adaptation of the key metabolic pathways during nutrient deprivation in liver and skeletal muscle, leading to an altered “fed state” metabolism, remaining the use of carbohydrates instead of fatty acids. As a consequence, the liver decreases the glycogen stores and accumulates triacylglycerol, while becoming hypergluconeogenic to supply enough glucose for muscle energy demands.

Since CPT1C seems to be constitutively inactive and would be activated under specific nutritional stressors, also considering its location in the endoplasmic reticulum together with a specificity for acyl-CoA substrate. We hypothesize that CPT1C is a key enzyme for sensing the pool of MBH acyl-CoAs and for triggering the adequate adaptive response from hypothalamus to peripheral tissues.

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O-16

INVOLVEMENT OF SSAO/VAP-1 IN OXYGEN-GLUCOSE DEPRIVATION-MEDIATED DAMAGE USING THE ENDOTHELIAL HSSAO/VAP-1-EXPRESSING CELLS AS EXPERIMENTAL MODEL OF CEREBRAL ISCHEMIA

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Vascular adhesion protein 1 (VAP-1) is a pro-inflammatory protein that mediates leukocyte recruitment through its semicarbazide-sensitive amine oxidase activity (SSAO, E.C 1.4.3.21). Plasmatic SSAO activity predicts the appearance of parenchymal hemorrhages after tissue plasminogen activator treatment in ischemic stroke patients, and it is increased as well in hemorrhagic stroke patients. The aim of this work has been to elucidate the role of SSAO/VAP-1 present in endothelial cells during ischemic stroke conditions. Based on the use of endothelial cells expressing or not the human SSAO/VAP-1 protein, different oxygen-glucose deprivation (OGD) and reoxygenation conditions were used as experimental approaches of stroke process. We found SSAO/VAP-1 expression increases the susceptibility of endothelial cells to OGD, and that its enzymatic activity, through specific substrates oxidation, increases the vascular cell damage under these experimental conditions. Caspase-3 and caspase-8 are activated during the death process. In addition, OGD constitutes a stimulus for the soluble SSAO/VAP-1 release, found elevated in many pathological conditions including stroke. Short-time OGD induces SSAO/VAP-1-dependent leukocyte binding on endothelial cells, partly dependent on its enzymatic activity, which may have consequences in progression of ischemic stroke. Furthermore, an indole substituted hydrazine JL72 and some MTDL molecules with a propargyl moiety, such as PF 9601N, ASS234 and DPH-4, besides their neuroprotective effects widely reported, show protective effects under selected experimental stroke conditions. These results show that SSAO/VAP-1 could participate in some of the processes occurring during stroke and this model could be a useful tool for screening new molecules as therapeutic agents for cerebral ischemia.

O-17

EFECTOS SEXO-DIMÓRFICOS DEL ESTRÉS TEMPRANO EN LA ATRIBUCIÓN DE SALIENCIA INCENTIVA EN LA ETAPA ADULTA EN RATAS

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En humanos, la exposición a situaciones estresantes en la infancia tiene efectos a largo plazo sobre el comportamiento y el SNC, constituyendo uno de los principales factores de riesgo para desarrollar psicopatologías. Las experiencias estresantes tempranas (ELS) en roedores tienen efectos a largo plazo que son parcialmente mediados por cambios en el cuidado materno. Las ELS pueden alterar el valor asignado a un estímulo incluyendo aquellos que predicen la aparición de recompensa, haciendo que adquieran un valor gratificante.

Se utilizaron ratas de ambos sexos y el ELS consistió en reducir el material para hacer el nido, midiéndose el cuidado maternal espontáneo. En la etapa adulta a la mitad de los sujetos se les expuso a un estrés agudo por inmovilización (IMO) para estudiar la posible interacción de este tratamiento con el ELS. Posteriormente se inició la medida de la saliencia incentiva hacia claves predictivas de recompensa, conducta que se ha asociado a una mayor vulnerabilidad a la adicción.
Los resultados indican que las madres realizan conducta materna compensatoria. En la etapa adulta las hembras sometidas a ELS muestran una reducción en la atribución de saliencia incentiva, indicativo de un efecto protector del ELS que se asoció a una disminución en la expresión del enzima sintético de dopamina (tirosina hidroxilasa) en el área tegmental ventral. Los machos se mostraron insensibles a este efecto. El tratamiento adulto de IMO fue inefectivo. El aumento compensatorio de la conducta materna puede ser el causante de este efecto sexo-dimórfico protector del ELS.

O-18

ESTUDI DE LA FISIOPATOLOGIA DE LA PRODUCCIO DE PUNTS GALLET MIOFASCIALS EN RATOLINS

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Una anòmala alliberació de l'acetilcolina (ACh) a la fenedura sinàptica sembla ser responsable de l'aparició de contractures localitzades sota del contacte sinàptic. Són els anomenats "nusos de contracció" i l'existència d'un nombre suficient d'ells es considera un punt gallet miofascial identificable mitjançant la palpació i / o electromiografia en els éssers humans.
Objectiu: La inducció de nusos de contracció per l'augment de l'acetilcolina en la fenedura sináptica en músculs de ratolins.
Metodologia: Es va augmentar la ACh en la fenedura sinàptica amb l'anticolinesteràsic neostigmina tant *in vivo* com *ex vivo*. Pels experiments *in vivo*, la neostigmina s'injecta per via subcutània en ratolins Swiss mascle adults. Hem utilitzat el múscul *elevador auris longus* (LAL) i el diafragma. Es van realitzar registres intracel·lulars de l'alliberament espontani d'ACh.
Resultats principals: Quan la neostigmina s'aplica per via subcutània (*in vivo, in toto*): l'alliberament espontània ACh es va incrementar fortament (~ 300%, P <0,05) en el múscul LAL, però no tant en el diafragma (~ 50%, p <0,05). Quan es va afegir la neostigmina en el bany de la càmera de registre (*ex vivo*), l'alliberament espontània ACh es va augmentar moderadament (~ 50%, p <0,05) en el múscul LAL però bastant mes al diafragma (~ 200%, P <0,05).
CONCLUSIONS. Les injeccions subcutànies de neostigmina indueixen un increment de l'alliberament espontani de ACh i, possiblement, podria ser utilitzat com un model per estudiar els punts gallet miofascials.

Aquest treball va ser recolzat per una beca de FISS (PI13 / 02084)

O-19

DECODING WIRING SPECIFICITY IN THE FLY VISUAL SYSTEM.

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How neurons select their synaptic partners and form specific connections has been a longstanding question in neurobiology. Our hypothesis is that molecular differences between closely related neurons with distinct wiring specificity would generate their unique connectivity patterns. Thus, we profiled the transcriptomes of two classes of *Drosophila* photoreceptor neurons, R7 and R8, and three classes of lamina neurons, L3, L4 and L5. These neurons elaborate distinct patterns of connections in the medulla, a multilayered neuropil of the optic lobe. Cells were isolated using fluorescence activated cell sorting during pupal development, prior to the extension to their specific final target layers. Several hundred genes appear differentially expressed (>5x) between these neurons in pairwise comparisons. Cell surface molecules (CSM) appeared enriched among the differentially expressed genes, consistent with their role as final effectors in cell-cell interactions. Comparisons of one neuron to all the others in our study identified CSM specific to each neuronal cell type. Among these cell-type specific CSM were members of protein families (i.e. Dpr, Beat, etc), with distinct sets of paralogs expressed in each cell type. We validated protein expression of several of these genes. The layer localized expression patterns of some of these genes is suggestive of their roles in wiring specificity. We also performed co-expression network analysis and found distinct networks for each type of neuron. In these networks a few transcription factors co-clustered with an array of CSM among other types of genes. These findings outline the genetic programs linking wiring specificity effectors to cell type specific transcriptional regulatory programs.

O-20

IDENTIFICATION OF GENES INVOLVED IN PHOTORECEPTOR WIRING SPECIFICIT

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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 postsynaptic partner among many different cells. Little is known about the mechanisms governing the specificity of synaptic connections in any system. Our hypothesis is that the molecular differences that exist between neuronal subtypes, with similar developmental origin and function, determine synaptic specificity. To identify these determinants we study two closely related neurons, the 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 synaptic layers during pupal development. Taking advantage of such precise coordination, we have their profiled their transcriptomes before, during extension and after reaching their temporary layers. The later time points coincide with the onset of synaptogenesis. Our bioinformatics analysis has identified a striking number of differentially expressed genes between the R7 and the R8 cells at the stages analyzed. We are screening candidate genes with genetic tools that allow us to simultaneously detect layer selection and synapse formation defects. Additionally, we are exploring the role of R8 and R7-specific transcription factors and the cis-regulatory regions of differentially expressed genes to gain insight into the transcriptional strategies that contribute to the R7 and R8 specific connectivity patterns.

O-21

HIGH-THROUGHPUT GENE EXPRESSION ANALYSIS OF HUMAN STRIATAL NUCLEI DEVELOPMENT IN CONTROL AND HUNTINGTON'S DISEASE PATIENTS

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Cell therapy is a key strategy in regenerative medicine to replace damaged tissues, and is a viable approach for treating Huntington's disease (HD) is characterized by specific degeneration of striatal GABAergic medium spiny neurons (MSNs) occurs. Implementation of cell replacement strategies for HD using pluripotent stem cells (PSC) requires development and characterization of new differentiation protocols since the available protocols are too long and inefficient. This can be partially explained because striatal developmental biology is quiet unknown and based on rodent developmental studies.
Our present aim is to establish specific human gene expression profile that can be used for the refinement of MSN differentiation protocols.
In this view, we analyze quantitative expression of 112 genes involved in telencephalon development, subpallium specification and striatal maturation in human fetal and adult tissues. We also compare post-mortem caudate and putamen nuclei of control and HD patients in order to establish differential expression gene pattern to be used as baseline in *in vitro* disease models.
Unbiased hierarchical cluster analysis reveals human whole ganglionic eminence (WGE) specific gene expression profile compared to fetal cortex and to adult striatal nuclei. Further analysis show a specific developmental pattern of expression in those genes involved in striatal specification. Expression levels comparison of control human tissues against diseased samples suggests specific pathways impairment.
In conclusion, we present a new quantitative dataset which spread new light on human striatal nucleus development at gene expression level. Interestingly, this dataset can be used as a baseline to evaluate the efficiency of available and future differentiation protocols helping refinement and evaluating the effects of modifications when improving. Furthermore, this approach may identify gene expression profiles that reflect HD-specific phenotypes.

O-22

PAPER DE P75^{NTR} DURANT EL NEURODESENVOLUPAMENT EN LA MALALTIA DE HUNTINGTON

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La malaltia de Huntington (MH) és un desordre neurològic caracteritzat per una pèrdua selectiva de neurones GABAèrgiques de projecció del nucli estriat. Com en altres processos neurodegeneratius, la investigació entorn la MH s'ha enfocat en definir els processos biològics implicats en la disfunció i mort neuronal en l'etapa adulta. No obstant, nombroses evidències suggereixen que la patologia observada en l'adult podria tenir el seu origen durant el desenvolupament. Diversos estudis han demostrat que el receptor de neurotrofines p75^{NTR} presenta una elevada expressió en determinades poblacions neuronals durant el desenvolupament, modulant la seva diferenciació i maduració, i disminuint un cop les neurites han contactat amb les cèl·lules diana. Per altra banda també es coneix que p75^{NTR} augmenta en condicions patològiques, tal i com nosaltres hem demostrat en models cel·lulars i murins de la MH. En aquest estudi hem avaluat el paper de p75^{NTR} durant la diferenciació i maduració de les neurones estriatals. Cultius primaris estriatals de ratolí demostren que la sobreexpresió de p75^{NTR} promou significativament el creixement dendrític en neurones provinents d'embrions salvatges (*wild-type*) mentre que en aquelles provinents d'embrions *knock-in* HdhQ111 (model de la MH) l'increment del creixement dendrític és significativament menor. Aquestes dades confirmen l'acció tròfica de p75^{NTR} en el neurodesenvolupament però a més suggereixen que la presència de la huntingtina mutada altera els mecanismes que participen en el creixement dendrític mediat per p75^{NTR}.

Comprendre els mecanismes moleculars activats per p75^{NTR} durant el neurodesenvolupament de l'estriat aportarà dades fonamentals pel disseny de noves estratègies terapèutiques duals que activin les vies tròfiques mediades per p75^{NTR} però antagonitzin la seva funció patològica en la MH.

Aquest treball ha estat finançat per ajudes del Ministerio de Economía y Competitividad (SAF2012-39142 a S. Ginés, SAF2011-29507 a J. Alberch), la Cure Huntington's Disease Initiative (CDHI), el Centro de Investigaciones Biomédicas en Red sobre Enfermedades Neurodegenerativas (CIBERNED CB06/05/0054 i CB06/05/0042) i el Fondo de Investigaciones Sanitarias Instituto de Salud Carlos III (RETICS: RD06/0010/0006).

O-23

ROLE OF MEMBRANE MICRODOMAINS IN NETRIN-1-DEPENDENT GROWTH CONE CHEMOREPULSION

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Netrin-1 is a bifunctional laminin-related guidance cue involved in axon pathfinding and cell migration processes during the development of the nervous system. Netrin-1 receptors of the Deleted in Colorectal Cancer (DCC) family are implicated in attraction and those of the UNC5 family in repulsion. Moreover, these proteins are also involved in cell death signaling, as they are also described as putative tumor suppressors whose expression is lost in numerous cancers. Biochemical assays identified some unc5 members to be located in specialized cholesterol-enriched membrane microdomains named lipid rafts. Here we assess mobility within the cell membrane of all four unc5 members (UNC5A, B, C, D) in HEK293T cells with a technique called FRAP, based in quantifying the amount of a fluorescently tagged protein that freely diffuses within the cell membrane after irreversibly photobleaching. This quantitative methodology let us appreciate differences among the UNC5 members not previously reported. Netrin-1 is directly involved in controlling navigation of axons from External Granular Layer (EGL) neurons during postnatal cerebellar development, and the presence of UNC5 confers the chemorepulsive behavior of EGL growth cones against Netrin-1. The combination of different approaches that selectively remove cholesterol from membranes and thus disorganize raft microdomains, let us appreciate that this raft localization of UNC5 proteins is required for Netrin-1-mediated axon repulsion and growth cone collapse. As a consequence, the presence of UNC5 Netrin-1 receptors in specialized raft microdomains appears to be crucial for the growth cone response to the chemorepulsive guidance cue Netrin-1.

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O-24

EPIGENETIC ANALYSES OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND INFLAMMATORY RELATED GENES IN ANXIOUS-DEPRESSIVE SPECTRUM DISORDERS: A TWIN BASED STUDY

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Anxious-depressive spectrum disorders hold the highest prevalence among mental disorders worldwide. They arise from the interplay between genetic and environmental risk factors, especially through early exposure to psychosocial stress (Aguilera et al. 2009). Hypothalamic-pituitary-adrenal (HPA) axis mediates stress response and has been described to be deregulated in anxious-depressed patients; this alteration seems to lie upon glucocorticoid receptor (GR) desensitization; likewise, cytokines and other pro-inflammatory mediators' levels in plasma are increased in depressed patients.
Epigenetic modifications can mediate environmental effects upon DNA sequence and further gene expression. Specifically, DNA methylation upon cytosines located in CpG dinucleotides of gene promoter regions is one of the most studied epigenetic signatures in mental disorders. **Aim.** To analyze the DNA methylation status of twelve HPA axis and inflammatory related genes in subjects informative for anxious-depressive condition.
Methods. We examined DNA extracted from whole blood from 34 subjects (17 MZ twin pairs), informative for psychopathological status, by means of the Illumina Infinium Human Methylation 450 Beadchip Kit. 203 CpG sites across the sequence of the twelve candidate genes (*CRHR1*, *NR3C1*, *FKBP5*, *PTGES*, *PTGS2*, *IL1B*, *IL6*, *TNF*, *IFNA*, *CRP*, *IL10* and *ILR1N*) were included in the association analysis. **Results and Discussion**. In agreement with previous findings, our results reveal a subtle association between *FKBP5* methylation pattern and anxious-depressive status (Klengel et al. 2013). Other genes such as *TNF*, *IL6* and *NR3C1* were hypomethylated in affected subjects. Further replication studies are required to corroborate and extend our results. **Acknowledgements.** ERA-NET NEURON Project (PIM2010ERN-00642), Comissionat per a Universitats i Recerca del DIUE (2014SGR1636).

O-25

THE NEURITIN 1 GENE IN SCHIZOPHRENIA SPECTRUM AND BIPOLAR DISORDERS AND ITS GENE-GENE INTERACTIONS

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Chromosome 6p25-p22 was identified by linkage studies as candidate for psychosis liability. It includes Neuritin-1 gene (*NRN1*), involved in neurodevelopment and plasticity processes and regulated by Brain-Derived Neurotrophic Factor (*BDNF*). Also in this region there is Dysbindin-1 gene (*DTNBP1*), a modulator of the synaptic transmission. We aimed to investigate: i) the association of *NRN1* with the risk for Schizophrenia Spectrum and Bipolar Disorders (SSD and BPD), ii) the interaction of *NRN1xBDNF* and *NRN1xDTNBP1* on the risk for these disorders.

Methods: Sample comprised 954 SSD-BPD patients and 715 healthy subjects. Eleven SNPs in *NRN1* (SNP1-SNP11:rs2208870-rs12333117-rs582186-rs645649-rs582262-rs3763180-rs10484320-rs4960155-rs9379002-rs9405890-rs1475157), one in *BDNF* (rs6265) and three in *DTNBP1* (SNP1-SNP3:rs2619537-rs2743864-rs1047631) were genotyped. Association analyses were conducted with UNPHASED and PLINK. *GenexGene* interaction was tested using logistic regression (SPSS-21) and MB-MDR.

Results: The frequency of the haplotype C-C(SNP4-SNP5) was significantly increased in patients (p=0.0043) while several haplotypes including SNP6-SNP11 were more frequent in controls ($p=6.8x10^{-5}$). *NRN1* was identified as having a statistical epistatic effect with *BDNF-Val/Val* genotype (p<0.009) and *DTNBP1* (SNP2_Acarriers, p=0.033).

Discussion: Our findings suggest that *NRN1* variability is a shared risk factor for both SSD and BPD. Moreover, the significant epistasis observed between *NRN1*x*BDNF* and *NRN1*x*DTNBP1* indicates the putative combined effect of both genes on the risk for SSD. Although the precise mechanism underlying these interactions are still unclear, it is interesting to note that the three genes are involved in neurodevelopment and plasticity, which are key processes in the SSD pathophysiology.

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O-26

ANALYSIS OF THE INFLUENCE OF *RGS4* AND *DAOA* GENES ON THE RISK FOR SCHIZOPHRENIA-SPECTRUM AND BIPOLAR DISORDERS AND THEIR ASSOCIATED COGNITIVE DEFICITS

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Background: Genome-wide association studies (GWAS) have provided evidence for a substantial shared polygenic component across schizophrenia-spectrum disorders (SSD). Several of the implicated genes have a role on glutamatergic neurotransmission, such as *D-amino Acid Oxidase Activator (DAOA)* and *Regulator of G-protein Signaling 4 (RGS4)*. We aimed to examine i) the relationship between *DAOA* and *RGS4* and risk for SSD, and ii) whether variation in these genes modulate patients' cognitive performance.
Methods: The family-based sample consisted of 753 subjects (222 patients and 531 first-degree relatives). Six SNPs in *DAOA* gene and 5 in *RG*
S4 were genotyped using Taqman-5'-exonuclease assays. Executive functions were assessed with Trail Making Test A and B (TMT-A/B) and Wisconsin Card Sorting Test (WCST). Genetic association analyses were conducted with PLINK, following a Transmission Disequilibrium Test (TDT).
Results: The haplotypes GAGACT at *DAOA* and TTGGA at *RGS4* were undertransmitted to SSD patients (p=0.007 and p=0.014, respectively), indicating its association with these disorders. In reference to *DAOA* and cognitive performance, the haplotype GAGGTT was related to better scores in TMT-B (p=0.019) and the GAGATT to worse scores in WCST (p=0.001). The TTAAA at *RGS4* was associated to a more impaired performance in TMT-A/B (p=0.041/p=0.046).
Conclusion: Our findings suggest that these genes may contribute to the risk for these disorders, as well as to modulate cognitive functioning, probably acting through a dysregulation of the glutamatergic signalling, among other processes.
Acknowledgements: CIBERSAM, ERA-NET NEURON Project (PIM2010ERN-00642), Comissionat per a Universitats i Recerca del DIUE (2014SGR1636).

O-27

FURTHER EVIDENCE THAT THE TYROSINE KINASE RECEPTOR DDR1 IS INVOLVED IN SCHIZOPHRENIA

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We found a genetic association between the Discoidin Domain Receptor 1 (*DDR1*) gene and schizophrenia and that DDR1 rs1264323 and rs2267641 were associated with the expression of its isoforms b and c in human brain. Furthermore, we reported that rs2267641 located within A2RE sequence, influences *DDR1* mRNA transport and alternative splicing.
A case-control study was carried out on patients with schizophrenia and healthy control subjects. A schizophrenia family sample (schizophrenia patients, relatives and control subjects) was also tested to assess the DDR1 SNP variation effect on cognitive speed processing.
Here we show that the rs1264323 T allele frequency is increased in the schizophrenic group ($P<0.027$) and the rare rs2267641 C allele was similarly distributed among the two groups. Interestingly, in the schizophrenia patient group we found that rs1264323 T associates with higher TMTA scores. Conversely, rs2267641 C statistically associates with lower TMTA scores ($P<0.02$). To check whether this genotype effect is exclusively present in patients, we explored relatives of patients and a control sample. The tendency of rs1264323 TT carriers to show higher TMTA scores was also present in relatives and in controls. However, rs2267641 CC effect on TMTA scores was not observed in the relatives and control groups.
We found that rs1264323 was associated with schizophrenia further evidencing the association between *DDR1* and the pathology. We could not replicate the association of rs2267641 with the disease. However, we found an association between rs2267641 and the cognitive speed processing only in schizophrenia patients, suggesting that an epigenetic phenomenon is driving in patients.

O-28

ANTIDEPRESSANT TREATMENT IS ASSOCIATED WITH LOW LEVELS OF HIGH-SENSITIVITY C-REACTIVE PROTEIN DURING THE EARLY STAGES OF PSYCHOSIS

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Prodromal schizophrenia patients have increased blood and central nervous system concentrations of inflammatory markers. Likewise, neuroinflammation has been linked to the pathogenesis of different neurodegenerative and psychiatric disorders. Different authors have proposed that antidepressants may possess central and peripheral anti-inflammatory properties. Interestingly, in early psychosis patients depressive symptoms are highly prevalent, and the association between depressive symptoms and inflammatory markers has not been explored. Also, there is scarce information about whether antidepressant treatment reduces the levels of inflammatory markers in these patients. Though we explored these relationships hypothesizing that depressive symptoms are associated with a higher degree of inflammation and that antidepressant treatment is associated with a reduction in the levels of inflammatory markers. We measured interleukin (IL)-6, high-sensitivity C-reactive protein (hs-CRP) and fibrinogen in 77 psychotic disorder (PD) patients with an illness duration of less than 5 years, treated (n=12) or not treated (n=65) with antidepressants, and in 25 healthy control (HC) subjects. The severity of psychotic symptoms was measured using the Positive and Negative Syndrome Scale (PANSS). Depressive symptoms were evaluated using the Hamilton Depression Rating Scale (HDRS) and the Calgary Depression Scale for Schizophrenia (CDSS). We observed that PD patients treated with antidepressants had lower hs-CRP levels compared with PD patients without antidepressant treatment and HC subjects (p=0.001). This difference could not be attributed to confounding variables (p=0.004). Our results suggest that antidepressants may constitute a potential anti-inflammatory treatment for schizophrenia.

O-29

LOWER MITOCHONDRIAL DNA CONTENT AND HIGHER NUMBER OF MITOCHONDRIA-RELATED CONDITIONS IN AUTISM SPECTRUM DISORDERS AND INTELLECTUAL DISABILITY COMPARED TO HEALTHY CONTROLS

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The causative factors involved in autism spectrum disorders (ASD) and intellectual disability (ID) are quite varied, may interact, and likely are also involved in other comorbid medical problems present in these patients. Moreover, clinical manifestations commonly associated to mitochondrial disorders are often present in ASD and ID. Therefore, the mitochondrial dysfunction hypothesis and the mitochondrial DNA (mtDNA) have been proposed as a transversal mechanism that can operate in ASD and ID. This study investigated the presence of conditions commonly associated to mitochondrial disorders in ID and ASD compared to healthy controls by analysing three study groups: a) patients fulfilling criteria for ASD and ID (N=122); b) patients fulfilling criteria for ID but not ASD (N=115); and c) healthy controls (N=42). Also, we measured, by quantitative real-time PCR, the mtDNA content of two mtDNA genes (*MT-ND1* and *MT-ND4*) in peripheral blood mononuclear cells. Either, patients with ASD+ID and patients with ID showed significantly a higher frequency of conditions commonly associated to mitochondrial disorders when compared to the controls: constipation, edema, seizures, eye disorders, sphincters incontinence, pregnancy and birth complications, and impaired ambulation. Moreover, ID patients showed lower *MT-ND1* content than the controls (25.7 and 32.3 copies/cell, respectively; $F=9.275$; $p<0.0001$), and both ID patients and ID+ASD patients showed lower *MT-ND4* when compared to controls (17.3, 19.5, and 17.3 copies/cell, respectively; $F=9.275$; $p<0.0001$). The present study adds further evidence for the mitochondrial involvement in ASD and ID, however further studies are needed to elucidate its specific role in each condition.

O-30

DESIGN OF PHOTOSWITCHABLE INHIBITORS OF B-ARRESTIN /B-ADAPTIN 2 INTERACTION: PEPTIDE FLEXIBILITY IS NOT A LIMITATION

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Protein-protein interactions (PPIs) are crucial for biological function and constitute interesting therapeutic targets. Many PPIs are mediated by short linear peptides, often α-helical. Molecules mimicking these peptides have been used to inhibit the PPIs that they mediate. However, photoswitchable peptides with little secondary structure have recently been developed as modulators of clathrin-mediated endocytosis. We used a panel of 14 azobenzene-crosslinked peptides from the β-arrestin BAP-long sequence to assess the relevance of secondary structure in their interaction with β-adaptin 2, and to identify the requirements for the design of photoswitchable inhibitors of PPI (PIPPIs). We assayed several crosslinking positions and distances and tested two kinds of non-proteinogenic amino acids as design tools. Flexible structures show greater inhibitory capacity over the observed PPI and enhanced ability to photoswitch than rigid structures. Absence of strong stabilization of helical structures in free inhibitor peptide is not a limitation in the design of PIPPI candidates.

O-31

CELLULAR SIGNALING IN EPO-STIMULATED NEUROBLASTOMA CELLS (SH-SY5Y)

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The hormone erythropoietin (EPO) has a well-described role in the homeostatic response against hypoxia. In addition to erythroid progenitors, its receptor has been found in neurons and could play a similar role in neuroprotection and defense against brain hypoxia. The objective of the present study is to analyze the effects of EPO stimulation on cultured SH-SY5Y cells, expressing the EPO receptor. After stimulation of confluent cultures with EPO, cells were extracted at different times. A double strategy was conducted with the resulting lysates: western blot analysis to evaluate total and phosphorylated FOXO3A; and HPLC analysis to characterize changes in signaling nucleotides (cAMP and cGMP), nucleotides related to cell metabolism (ATP, ADP and GTP) and soluble phosphorylated inositols (InsP_1, InsP_2 and InsP_3).

Our preliminary results show that EPO induces rapid changes in soluble phosphorylated inositols (around 1 minute) and slow ones in FOXO3A phosphorylation levels (several minutes) in neuroblastoma cells. Moreover, we identified different inositol isomers in SH-SY5Y, as previously described in the neuronal line GH3. Our novel observations imply that the Ins(1,4)P_2 isomer is more sensitive to EPO stimulation than the Ins(2,4)P_2, its relative levels increasing after 2 minutes of stimulation. As well, nucleotide analysis results indicate a tendency to increasing cGMP after 2 minutes of stimulation, and no effect was observed in cAMP levels. No changes in ATP, ADP or GTP were observed in the conditions at which cells were cultured in the stimulated groups versus controls, indicating that cells were in a good metabolic status when stimulated.

O-32

CREB REGULATES CALCIUM EXCITABILITY IN ASTROCYTES VIA MITOCHONDRIA

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Astrocytes are calcium-based excitable cells that modulate neurotransmission, learning and memory; although how they do it is not completely understood. Our working hypothesis is that experience induces CREB-mediated long-lasting changes in astrocytes that, in turn, modulate the activity of astrocyte-neuronal circuitries. In this study we have asked the straight question of whether CREB modulates the gliotransmitter-elicited increases in cytosolic calcium in rat cortical astrocyte cultures. To do so, CREB-dependent transcription was triggered by 1 hour-pulses with noradrenaline (10μM) or ATP (100μM), which we have previously shown that activate CREB-dependent transcription in astrocytes within 6 hours, and cytosolic calcium responses were assessed by calcium imaging in Fluo-4 loaded astrocytes challenged by gliotransmitters (ATP, NA and ET-1). Agonist-induced calcium responses were lowered 15-38% and this reduction was reverted by the viral transduction of a dominant negative form of CREB (A-CREB). Likewise, viral transduction of a constitutively active form of CREB (VP16-CREB) caused a similar decrease in gliotransmitter-induced calcium response, as compared to astrocytes infected with an empty virus (Null). The CREB-induced decrease in calcium transients was still observed in the presence if EGTA but not if mitochondrial calcium uptake was blocked by the mitochondrial proton gradient uncoupler FCCP. In agreement, ATP-induced mitochondrial calcium rises, directly monitored with Rhod-2, were around 3 times higher in VP16-CREB overexpressing than in Null astrocytes. In conclusion, our results show that CREB changes calcium excitability in astrocytes by altering calcium compartmentalization between cytosol and mitochondria.

O-33

ESSENTIAL ROLE OF THE PDK1 SUBSTRATE-DOCKING SITE IN REGULATING NEURONAL POLARIZATION, AXON OUTGROWTH, NEURONAL MIGRATION AND CORTICAL LAYERING

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The phosphoinositide 3-kinase (PI 3-kinase)/Akt signaling pathway plays essential roles during the development of the central nervous system. The 3-phosphoinositide-dependent protein kinase 1 (PDK1) coordinates the PI 3-kinase signals by phosphorylating and activating twenty three kinases of the AGC family. Phosphorylation of a conserve docking site in the substrate is a requisite for PDK1 to recognize and activate most of these kinases, with the exception of PKB/Akt. We exploited this differential mechanism of regulation by generating neuronal-specific conditional knock-in mice that express a mutant form of PDK1 in which the substrate-docking site binding groove, termed PIF-pocket, has been disrupted. In neuronal tissues and cells derived from these mice, activation of all the PDK1 substrates tested with the exception of PKB/Akt was severely impaired. As a consequence, mice exhibited microcephaly, aberrant patterns of layering in the cortex and reduced connectivity of particular circuits, leading to specific neurobehavioral disorders. The abnormal patterning of the adult brain arise from the reduced ability of the embryonic neurons to polarize, generate axons and migrate, therefore highlighting the important role that the other kinases regulated by PDK1 beyond PKB/Akt play in mediating essential neuronal responses dictated by PI 3-kinase that are instructive for brain development.

O-34

CANVIS D'ACTIVITAT-DEPENENT SOBRE LA ISOFORMA NPKCε A TRAVÉS DE LA FUNCIÓ DEL TRKB EN LA UNIÓ NEUROMUSCULAR DE RATA ADULTA

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Protein kinase C (PKC) is a family of protein kinase enzymes that are widely distributed in all cells and at high concentrations in neural tissues and regulate a variety of neural functions including neurotransmitter release. Thus, identifying the mechanisms that activate and compartmentalize PKC isoforms is fundamental to extend knowledge about the physiological role of individual PKC isoforms.

The present study is designed to examine the location of the novel isoform nPKCε at the neuromuscular junction (NMJ), its synaptic activity-related expression changes and its regulation by muscle contraction. We used immunohistochemistry and confocal microscopy to demonstrate that nPKCε is exclusively located in the motor nerve terminals of the adult rat NMJ. We also report that electrical stimulation of synaptic inputs to the skeletal muscle (1 Hz for 30 minutes) significantly increased the amount of the nPKCε in the synaptic membrane. Moreover, we found that muscle contraction is necessary for nPKCε expression changes.

The results also demonstrate that synaptic activity-induced muscle contraction promotes changes in nPKCε through tyrosine kinase receptor B (TrkB) signaling pathway. Together, these results provide a mechanistic insight into how synaptic activity-induced muscle contraction could regulate the presynaptic action of nPKCε. These findings suggest that muscle contraction is an important regulatory signaling step in nPKCε expression through TrkB at the NMJ.

O-35

A PHOTOCHROMIC ALLOSTERIC MODULATOR TO CONTROL AN ENDOGENOUS G PROTEIN-COUPLED RECEPTOR WITH LIGHT

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Side effects of drugs are the major drawback for the use of any medicine in clinics, and depend mainly on small therapeutic windows and off-target actions. Obtaining a fine control over the temporal and spatial activity of a drug would allow precise dosing and localization of active drugs, respectively. These optimal therapeutic conditions cannot be achieved with any existing drug, but light can help solving the limitations of classical pharmacology.

Optopharmacology is a recently developed technique that consists of attaching a light-sensitive moiety (usually azobenzene) to ligands that control endogenous proteins, so that drugs can be made available with the spatiotemporal precision of light. Still, optopharmacology was never applied before to the most interesting targets for drug development: allosteric modulators of G protein-coupled receptors (GPCRs).

We report the synthesis and characterization of the first light-sensitive allosteric modulator of the metabotropic glutamate receptor 5 ($mGlu_5$), a class C GPCR, by a novel optopharmacological design.
Instead of assembling azobenzene next to the ligand, like in previous optopharmacology, we integrated azobenzene inside a known allosteric ligand of mGlu receptors by simple substitution of few atoms in the parent compound. One of the obtained molecules (Alloswitch-1) was active with nanomolar potency and light wavelength-specificity as a negative allosteric modulator (NAM) of $mGlu_5$. In cultured cells, Alloswitch-1 counteracts calcium oscillations induced by activation of both heterologous and endogenous mGlu5 receptors in the dark, and different light conditions can be used to switch these oscillations on and off reversibly. In Xenopus tropicalis tadpoles, Alloswitch-1 controls the motility of animals in a light- and concentration-dependent manner.

Alloswitch-1 is a novel and powerful tool for the optical control of an endogenous GPCR. Moreover, the uncommonly high success rate of our novel design strategy suggests that it can be extended to allosteric modulators of other GPCRs.

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O-36

HIPPOCAMPAL DECREASED MEF2C LEVELS ARE ASSOCIATED WITH COGNITIVE DYSFUNCTION AND BDNF REDUCTION IN HUNTINGTON'S DISEASE

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The family of transcription factors myocyte enhancer factor 2 (MEF2) has been recently involved in memory formation process. Cognitive deficits have been highlighted as an important component of the Huntington's disease (HD) related to hippocampal dysfunction. Therefore, we studied the role of MEF2 in the hippocampus of two HD mice models, the R6/1 an exon-1 mice model, and a full-length mice model, the knock-in mice (Hdh^{Q111}). The analysis of MEF2 protein levels at different ages revealed a decrease of these transcription factors in both mice models from the onset of cognitive dysfunction. The transfection of htt-94Q-GFP in the neural cells ($STHdh^{Q7/Q7}$) resulted in an important decrease of MEF2 protein levels. Moreover, we detected in hippocampal primary cultures infected with adenoviruses expressing mutant huntingtin (Ad128T-htt) that MEF2 colocalizes with the intracellular mutant huntingtin aggregates. Among the MEF2 genes expressed in the brain, we observed that the loss of MEF2 is associated with a decrease in MEF2c, with no changes in MEF2a protein levels. To know whether MEF2c is implicated in the reduction of hippocampal BDNF levels, an important hallmark of the HD, we transfected $STHdh^{Q7/Q7}$ cells with a positive form of MEF2c (mtMEF2C219). We observed that BDNF protein levels are enhanced in cells expressing mtMEF2C219. Our results suggest that the loss of MEF2c factor is involved in the reduction of BDNF that occurs in the hippocampus of HD.

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O-37

EFFECTS OF THIOREDOXIN AND GLUTATHIONE SYSTEMS RELATED TO MISFOLDING AND AGGREGATION OF hSOD1 MUTANTS

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Protein misfolding has been related to neurodegenerative diseases and in the case of the Amyotrophic Lateral Sclerosis (ALS) a wide variety of proteins have been associated to familial forms of this devastating disease, being the human Superoxide dismutase 1 (hSOD1) the first to be identified. Up to now, more than 100 mutations related to ALS have been described in hSOD1 with no apparent regional preference.

hSOD1 has an internal disulfide bond between C57 and C146 which presumably confers high structural stability under the cytoplasmic reducing conditions which are mainly supported by the thioredoxin(Trx) and glutathione (GS) systems. In this study, we have used wild type (WT) and point mutated (G93A and A4V) recombinant hSOD1 to analize the effect of GS and Trx in a reducing context. In addition, the RT4 cell line was transfected with the hSOD1-GFP constructs, and submitted to oxidizing intracellular conditions by inhibiting either the synthesis of GSH with buthionine sulfoximine (BSO) or the thioredoxin reductase (TrR) activity with aurothioglucose (ATG).

Results show that WT protein is insensitive to Trx system, while G93A and A4V are reduced either by GSH or Trx in a different extent. Moreover, cells transfected with hSOD1-G93A-GFP or hSOD1-A4V-GFP showed bright cytoplasmic fluorescent protein aggregates when treated either with BSO or ATG, in contrast to cells transfected with WT-hSOD1,.These results strongly support that mutations on hSOD1 induce protein misfolding, exposing the conserved disulfide bond that is normally hidden in the wild type, non-mutated, protein. Consequently. the misfolded hSOD1aggregates under oxidizing conditions because the exposed cysteines form new ectopic disulfide bonds. This work is supported by grants SAF2011-27566,SAF2011-28485 and SGR2009/152.

O-38

NUCLEAR MORPHOLOGY IS ALTERED IN HUNTINGTON'S DISEASE: ROLE OF LAMIN B

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Lamins are the major structural proteins inside the nuclear lamina and their alterations are implicated in relatively few diseases, classified as laminopathies. In addition, lamin alterations have been described as playing a role in some neurodegenerative diseases, such as adult onset autosomal dominant leukodistrophy and Parkinson's disease. We have recently described increased lamin B levels in in the putamen of Huntington's disease (HD) patients, as well as in the striatum of R6/1 mouse model of HD (Rue et al., 2014). Here, we extended our study by analyzing lamin B isoforms, B1 and B2, and also lamins A and C in several brain regions of R6/1 mice at different stages of the disease. We found that lamin B1 and B2 levels were increased in the striatum and cortex from early stages of the disease while in the hippocampus their levels were only augmented at late stages. Lamin A and C levels were also enhanced in the striatum and hippocampus at late stages, but they were not altered in the cortex. Immunohistochemical analysis showed a clear redistribution of lamin B1 in the nucleus, which presented irregular forms in R6/1 mice neuronal cells when comparing with control ones. Interestingly, similar alterations were detected in the brain of HD patients. Due to the role of lamin in chromatin organization, gene transcription and oxidative stress responses, our results suggest that these alterations could have important implications in the pathophysiology of HD. Thus, normalizing lamin protein levels could be a potential therapeutic strategy for this devastating neurodegenerative disease.

O-39

RTP801 IS INVOLVED IN MUTANT HUNTINGTIN-INDUCED CELL DEATH

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RTP801 is induced by cellular stresses and has a pro-apoptotic function in non-proliferating differentiated cells such as neurons. In several neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease, elevated levels of RTP801 have been detected, thus suggesting a role for RTP801 in neuronal death. Indeed, neuronal death is a pathological hallmark in Huntington's disease (HD), an inherited neurodegenerative disorder caused by the mutated huntingtin protein. Currently, the exact mechanisms underlying mutant huntingtin (mhtt)-induced toxicity are still unclear. Here we investigated whether RTP801 is involved in mutant htt-induced cell death. Ectopic exon-1 mutant htt elevated RTP801 mRNA and protein levels in NGF-differentiated PC12 cells. We also observed a significant increase of RTP801 protein in primary cortical neurons overexpressing exon-1 mhtt. A part from inducing RTP801 gene expression, we found that mutant htt contributed to RTP801 protein accumulation by reducing its proteasomal degradation rate. Interestingly, silencing RTP801 expression with shRNAs blocked mhtt-induced cell death in NGF-differentiated PC12 cells. Moreover, RTP801 protein levels were not altered in the striatum of $Hdh^{Q7/Q111}$ and R6/1 mice, two HD models that display motor deficits but no neuronal death. Importantly, RTP801 protein levels were elevated in the putamen and cerebellum of HD patients. Taken together, our results confirm RTP801 as a novel downstream effector of mhtt-induced toxicity and suggest that their relationship may be relevant to the human disease.

O-40

CRTC1 OVEREXPRESSION RESCUES B-AMYLOID-INDUCED HIPPOCAMPAL-DEPENDENT MEMORY DEFICITS IN APP TRANSGENIC MICE

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Cognitive decline is associated with gene expression changes in the brain, but the transcriptional mechanisms underlying memory impairments in cognitive disorders, such as Alzheimer's disease (AD), are largely unknown. We aimed to elucidate relevant mechanisms responsible for transcriptional changes underlying early memory loss in AD by examining pathological, behavioral, and transcriptome changes in control and mutant β-amyloid precursor protein ($APP_{Sw,Ind}$) transgenic mice during aging. Genome-wide transcriptome analysis using mouse microarrays revealed deregulation of a gene network related with neurotransmission, synaptic plasticity, and learning/memory in the hippocampus of $APP_{Sw,Ind}$ mice after spatial memory training. $APP_{Sw,Ind}$ mice show changes on a transcriptional program dependent on the CREB-regulated transcription coactivator-1 (CRTC1). Importantly, we found that synaptic activity and spatial memory induces CRTC1 dephosphorylation and activation leading to CRTC1-dependent transcription in the hippocampus, and these events are impaired in $APP_{Sw,Ind}$ mice at early pathological stages. Furthermore, CRTC1 overexpression in the $APP_{Sw,Ind}$ mouse hippocampus efficiently reverses Aβ -induced spatial learning and memory deficits by restoring a specific subset of CREB/CRTC1 target genes. Our results reveal a critical role for CRTC1-dependent transcription on spatial memory and provide evidence that targeting CRTC1 can reverse memory loss in AD.

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O-41

THE EFFECT OF HUNTINGTIN GENE CAG REPEAT EXPANSION ON HUMAN INDUCED PLURIPOTENT STEM CELL NEURONAL DIFFERENTIATION.

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Cell therapy is a key strategy in regenerative medicine to replace damaged tissues, and is a viable approach for treating Huntington's disease (HD) where specific degeneration of striatal GABAergic medium spiny neurons (MSNs) occurs due to CAG repeat expansion in the *htt* gene. Implementation of cell replacement strategies using pluripotent stem cells requires development and characterization of new differentiation protocols as previously described protocols are long and produce MSNs inefficiently.
In this study we use a recently developed protocol which generates a significant level of MSN-like neurons from human embryonic stem cells, and apply it to induced pluripotent stem cell (iPSC) lines derived from HD and control patients. We characterise progression through differentiation at the protein level by immunocytochemistry, and at the gene expression level using a customised qPCR platform that monitors expression of genes specific for neural developmental stages and/or encephalic areas. At the later stages of the protocol we assess neuronal functionality.
We observe clear differences in the rate that different iPSC lines progress through differentiation with each cell line displaying a different gene expression profile. Some iPSC lines produce neurons expressing key MSN markers to a lesser or greater extent, with calcium signaling imaging indicating that this protocol generates a high number of functional neurons.
Our results show that this differentiation protocol more rapidly generates MSNs at a level comparable to or better than previously described protocols. Also we observe that CAG repeat expansion alters the rate of iPSC differentiation and potentially influences MSN development and functionality.

O-42

ADENOSINE A1 AND A2A RECEPTOR HETEROMERS FORM DYNAMIC BUT STABLE TETRAMERIC COMPLEXES WITH TWO DIFFERENT G PROTEINS

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G-protein-coupled heteromers serve as unique protein complexes that allow cells to sense the environment in a variety of ways. The dynamics and structural characteristics underlying their functional diversity are not known. Studying the model heteromer of adenosine A_1 and A_{2A} receptors, we show here by single particle tracking experiments, that heteromers can form dynamic but stable heterocomplexes. Using biophysical energy transfer techniques and single molecule microscopy, together with molecular models of protein oligomerization, we provide experimental evidence to support a model of these A_1-A_{2A} receptor complexes to be heterotetramers formed by two transmembrane helix-4-interacting A_1 and A_{2A} homodimers bound together via transmembrane helix 5. The resulting non-square heterotetramer forms a complementary interface that can simultaneously accommodate two separately bound α β γ heterotrimeric G proteins (G_s and G_i) only if the γ but not α subunits face the inside of the heterotetramer.

O-43

PRESYNAPTIC CLATHRIN LEVELS ARE A LIMITING FACTOR FOR SYNAPTIC TRANSMISSION

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To maintain communication, neurons must recycle their synaptic vesicles with high efficiency. This process places a huge burden on the clathrin-mediated endocytic machinery, but the consequences of this are poorly understood. We found that the amount of clathrin in a presynaptic terminal is not fixed. During stimulation, clathrin moves out of synapses as a function of stimulus strength and neurotransmitter release probability, which, together with membrane coat formation, transiently reduces the available pool of free clathrin triskelia. Correlative functional and morphological experiments in cholinergic autapses established by superior cervical ganglion neurons in culture show that presynaptic terminal function is compromised if clathrin levels fall by 20% after clathrin heavy chain knock down using RNAi. Synaptic transmission is depressed due to a reduction of cytoplasmic and readily releasable pools of vesicles. However, synaptic depression reverts after dialysis of exogenous clathrin, thus compensating RNAi-induced depletion. Lowering clathrin levels also reduces quantal size, which occurs concomitantly with a decrease in the size of synaptic vesicles. Large dense-core vesicles are unaffected by clathrin knock down. Together, our results show that clathrin levels are a dynamic property of presynaptic terminals that can influence short-term plasticity in a stimulus-dependent manner.

O-44

TWO-PHOTON NEURONAL AND ASTROCYTIC STIMULATION WITH AZOBENZENE-BASED PHOTOSWITCHES

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Synthetic photoswitches developed by optochemical genetics and optopharmacology can be designed to control a variety of proteins and their biochemical functions with light, and have been extensively used in ion channels and receptors. However, the high spatiotemporal precision and tissue penetration of two-photon stimulation has never been investigated in these molecules. Here we use a light-gated glutamate receptor (LiGluR) to demonstrate two-photon excitation of azobenzene-based protein switches like the maleimide-azobenzene-glutamate (MAG) compound. We also present versatile strategies to enhance the photochemical response of MAG derivatives by (1) introducing an asymmetric aminoazobenzene with sufficiently strong push-pull character as to enhance its two-photon absorption cross-section, and (2) introducing a light-harvesting antenna to sensitize the trans-cis isomerization of the system by absorption of near-infrared radiation and subsequent resonant electronic energy transfer to the trans-azobenzene group. Using these compounds, we show that two-photon activation of LiGluR elicits action potentials in hippocampal neurons and triggers calcium-regulated processes in astrocytes with subcellular resolution.

In conclusion, we demonstrate the two-photon activation of azobenzene-based photoswitches using LiGluR and rationally designed MAG derivatives with visible absorption, fast thermal relaxation and high two-photon isomerization efficacy based on push-pull substitutions and sensitization of the azobenzene photoisomerization. These modifications allow adjusting the photochemical properties without altering protein function. The reported multiphoton excitation conditions should be directly applicable to all azobenzene-based bioactive ligands reported, including intracellular photoswitches. Our findings thus enable the use of synthetic photoswitches to manipulate extra- and intracellular biochemical processes with the spatiotemporal precision provided by two-photon stimulation.

O-45

CPT1C: NEW PROTEIN IN AMPAR COMPLEXES AS TRAFFICKING MODULATOR

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AMPARs mediate the vast majority of fast excitatory transmission in the brain. The biophysical and trafficking properties of AMPARs depend on their subunit composition, on posttranscriptional/posttraductional modifications and on auxiliary proteins present in the receptor complex. Despite the existence of numerous AMPAR partners, recent proteomic studies have exposed even more interacting proteins that could potentially be involved in AMPAR regulation. Amongst these, carnitine palmitoyltransferase 1C (CPT1C) has been demonstrated to form an integral part of native AMPAR complexes. Thus, we aimed to investigate whether CPT1C might be able to modulate AMPAR function. Firstly, we confirmed that both proteins interact in heterologous expression systems. Secondly, CPT1C enhanced whole-cell AMPAR currents in a GluA1 subunit-specific manner. However CPT1C does not alter the biophysical properties of GluA1 homomeric receptors and co-localization experiments revealed that the GluA1-CPT1C complex is not present at the cell surface. We checked whether a higher number of receptors at the plasma membrane could be responsible for the increase of the whole-cell currents, and our results confirmed an increase in GluA1 content at the plasma membrane in the presence of CPT1C. Surprisingly, despite the potential depalmitoylating action of CPT1C over this specific residue, we did not observe changes of the palmitoylation state of GluA1. To sum up, this study shows that CPT1C controls GluA1-containing AMPAR surface expression acting on cysteine 585, hence describing a novel regulatory function for the elusive CPT1C.

O-46

SPECIFIC REGULATION OF GLUA SUBUNIT SYNTHESIS AND AMPA RECEPTOR-MEDIATED SYNAPTIC FUNCTION BY CPT1C IN THE HIPPOCAMPUS

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Ionotropic glutamate receptors subfamily of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPARs) mediates most of the fast excitatory synaptic transmission in the central nervous system. The regulation of AMPARs abundance in the postsynaptic membrane is an important mechanism involved in learning and memory formation. Recent data suggest that one of the constituents of the AMPAR complex is carnitine palmitoyltransferase 1 C (CPT1C), a brain-specific isoform located in the endoplasmic reticulum of neurons. Previous results demonstrated that CPT1C deficiency disrupted spine maturation in hippocampal neurons and impaired spatial learning, but the role of CPT1C in AMPARs physiology remained completely unknown. In the present study we show that AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) and synaptic levels of AMPAR subunits GluA1 and GluA2 are greatly reduced in the hippocampal neurons of CPT1C knockout (KO) mice, and we demonstrate that AMPARs expression is dependent on CPT1C levels. Total GluA1 and GluA2 protein levels, but not those of other synaptic proteins, decreased in the hippocampal cultures of CPT1C KO mice and increased in CPT1C overexpressing neurons. Notably, mRNA levels of AMPARs remained unchanged, indicating that CPT1C is involved in GluA1 and GluA2 protein turnover. The synthesis of GluA1 after chemical long-term depression was clearly diminished in CPT1C-deficient neurons while the rate of protein degradation remained unaffected. In addition, we show that CPT1C binds GluA1 and GluA2 but no other auxiliary proteins. These data newly identify CPT1C as a regulator of AMPARs protein synthesis and synaptic function through direct interaction.

O-47

DYRK1A-MEDIATED PHOSPHORYLATION OF GLUN2A AT SER1048 REGULATES THE SURFACE EXPRESSION AND CHANNEL ACTIVITY OF GLUN1/GLUN2A RECEPTORS

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N-methyl-D-aspartate glutamate receptors (NMDARs) play a pivotal role in neural development and synaptic plasticity, as well as in neurological disease. Since NMDARs exert their function at the cell surface, their density in the plasma membrane is finely tuned by a plethora of molecules that regulate their production, trafficking, docking and internalization in response to external stimuli. In addition to transcriptional regulation, the density of NMDARs is also influenced by post-translational mechanisms like phosphorylation, a modification that also affects their biophysical properties. We previously described the increased surface expression of GluN1/GluN2A receptors in transgenic mice overexpressing the Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), suggesting that DYRK1A regulates NMDARs. Here we have further investigated whether the density and activity of NMDARs were modulated by DYRK1A phosphorylation. Accordingly, we show that endogenous DYRK1A is recruited to GluN2A-containing NMDARs in the adult mouse brain, and we identify a DYRK1A phosphorylation site at Ser^{1048} of GluN2A, within its intracellular C-terminal domain. Mechanistically, the DYRK1A-dependent phosphorylation of GluN2A at Ser^{1048} hinders the internalization of GluN1/GluN2A, causing an increase of surface GluN1/GluN2A in heterologous systems, as well as in primary cortical neurons. Furthermore, GluN2A phosphorylation at Ser^{1048} increases the current density and potentiates the gating of GluN1/GluN2A receptors. We conclude that DYRK1A is a direct regulator of NMDA receptors and we propose a novel mechanism for the control of NMDAR activity in neurons.

Keywords: GluN2A, DYRK1A, phosphorylation, NMDA receptor, trafficking, Down syndrome.

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P-0

MAPA DE LA RECERCA EN NEUROCIÈNCIES A CATALUNYA v2

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El mes de maig de 2009 vàrem preparar l'anomenat *"Mapa precolombí de la recerca en Neurociències a Catalunya"* on recollirem dades bàsiques de 94 grups de recerca en aquest camp. Vàrem anomenar-lo precolombí coneixedors de que tindria mancances per ser una primera versió. Així, per exemple, els grups de recerca en hospitals o els de neurociència conductual es trobaven infrarepresentats. Presentem ara una versió ampliada i actualitzada del mapa amb dades recollides al gener-febrer de 2014. Aquesta versió inclou dades de 116 grups de recerca amb >931 investigadors la qual cosa demostra l'existència d'una notable massa crítica d'investigadors en Neurociències a Catalunya. Les àrees principals de recerca d'aquests grups són Trastorns del sistema nerviós (72%), seguida de Mort neuronal/neuroprotecció (47%), Cognició i conducta (42%), Senyalització (34%), Neurotransmissió (32%), Cèl·lules glials/neuroinflammació (25%), Desenvolupament (19%) i altres (16%). Es manté l'atomització dels grups que ja vàrem detectar al 2009 de manera que la gran majoria de grups són de mida petita (≤ 5 membres; 45%) o mitjana (6-14 membres; 40%) i únicament el 15% dels grups (n=17) poden ser considerats de mida gran (≥ 15 membres). Es manté igualment la desproporció de gèneres pel que fa als caps de grups amb un 55% de grups liderats per homes, un 32% per dones i un 13% amb direcció mixta. Els grups identificats pertanyen a 25 institucions diferents sent la UAB, la UB i l'IDIBAPS les institucions amb més grups de recerca. Geogràficament, els campus amb un major nombre de grups són Bellaterra/UAB (30), Casanova (24), Diagonal (13), Bellvitge (10), Sant Pau (7), Mar/PRBB (7), Vall d'Hebron (5), Sant Joan de Déu (5) i Lleida (4). Creiem que aquest mapa aporta dades per a conèixer la realitat de la recerca en neurociències a Catalunya i alhora pot ser una eina útil per a investigadors (per a establir col·laboracions, identificar laboratoris on aprendre tècniques, on fer una tesi o un post-doc, etc...) i per a comunicadors científics (per identificar grups experts en temes específics). El nostre objectiu és actualitzar aquest mapa cada 2 anys tot coincidint amb el simposi de Neurobiologia experimental de la SCB. Podeu consultar-lo a http://taller.iec.cat/neurocat. Agraïm sincerament a tots els grups de recerca que han enviat les seves dades sense la participació dels quals aquest mapa no hagués pogut ser una realitat.

P-1

EARLY CHANGES IN DNA EXPRESSION CONTROL IN FEMALE SAMP8 EXPLAIN SENESCENT PHENOTYPE BY EPIGENETIC MODULATION

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Ageing and age-related cognitive impairments are becoming one of the most important issues for human health. Adult neurogenesis is directly implicated in brain ageing, and both seem to be controlled at the epigenetical level. This epigenetic mechanisms act during the development of the nervous system. Repressor complex REST inhibits the expression of neurogenic genes by binding to their cis-elements and by upregulating histone deacetylase (HDAC) activity. Moreover, neurogenic gene transcription is reduced by histone demethylation and deacetylation caused by repressor complex REST.
Here, we focus on the participation of the repressor complex REST in the senescence 1 month-old SAMP8 mice that shown characteristic associated to aging at an early age. SAMP8 mice display decreased NeuN staining and increased GFAP compared to age-matched SAMR1 mice. Together with this we reported a marked deficit in memory tasks for SAMP8 mice. REST and the components of REST-complex protein levels are increased at 1 month old SAMP8 in reference to SAMR1. LSD1 protein level increased was confirmed also by immunohistochemistry. Doublecourtin (DCX) protein level, a selective marker of cells committed to the neuronal lineage, was found diminished. Real-time PCR analysis showed changes in HDAC2 gene expression. Gene expression changes were found in BRAF35/HMG20b, an activating component of REST complex, and Neurod6 (REST-responsive gene). Overexpression of Neurod6 results in neural differentiation of murine embryonic stem cells. These evidences could be a preliminary clue about the involvement of epigenetic control mediated by REST complex in ageing and neurogenesis process.
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P-2

STUDY OF THE DYNAMICS OF UNC5 NETRIN 1 RECEPTORS WITHIN MEMBRANE MICRODOMAINS.

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Netrins are bifunctional guidance cues, they can attract or repel elongating axons, signalling through DCC and unc5 receptors. Unc5 family members (a-d) are known to be involved in repelling axons away from netrin 1 sources during nervous system development. Biochemical assays identified unc5 members to be located in specialised microdomains named lipid rafts which are cholesterol enriched microdomains within the cell membrane, known to be important for signal transduction among other cellular processes. In the present work we assess mobility within the cell membrane of all unc5 members in HEK293T cells with a technique named FRAP, based in quantifying the amount of a fluorescently tagged protein that laterally diffuses within the cell membrane after irreversibly photobleaching. We show that unc5c has the lowest M_f when it is compared with the other members. Moreover, we assess localization of unc5 receptors in lipid raft microdomains and in the membrane, by using specific membrane labelling. We provide evidences that unc5(b-d) can be found in the cell membrane and additionally in lipid rafts microdomains. Detailed analysis of unc5c expression evidences that, unlike other family members, is distributed in a punctate manner, exhibiting two different membrane dynamics, and that proteins can move in and out of those clusters, however, when the protein is located in a cluster, the mobility is reduced. We also show that dynamics of unc5b and unc5c are altered when membrane cholesterol is depleted. Finally, we show that deleting the death domain of unc5b and unc5c results in an increase of M_f of both receptors.

P-3

DIFFERENTIAL INVOLVEMENT OF THE JNK ISOFORMS IN THE CONTROL OF ADULT BRAIN.

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The c-Jun N- terminal kinases (JNKs) are members of the MAPK (Mitogen-Activated Protein Kinase) family. The activation of JNK signaling cascade is critical for occurring neuronal death. In addition, its activation regulates cell proliferation and migration. Multiple splice variants of JNKs encoded by three distinct genes have been identified: JNK1, JNK2 and JNK3. Using different adult knock-outs mice (*jnk1*$^{-/-}$, *jnk2*$^{-/-}$, *jnk3*$^{-/-}$), we detected that the lack of one of these isoforms, induced brain alterations in calcium binding proteins (CaBP), axonal myelination, axonal pattern and in the cells of the subgranular zone (SGZ). The CaBP immunolabelling revealed an increase in SGZ calretinin immature (CR-IR) neurons in *jnk1*$^{-/-}$ mice respect wild type (wt) mice, a general increase in calbindin-immunoreactivity (CB-IR) in the hippocampus of *jnk2*$^{-/-}$ mice and a decrease in *jnk3*$^{-/-}$ mice *versus* wt. In all knock-outs was noticed a decrease in MBP-immunoreactivity (Myelin Basic Protein) and specifically, in *jnk1*$^{-/-}$ mice, it was also shown a decrease in MAP2-immunoreactivity (Microtubule Associated Protein). DCX (Double Cortin Protein), that labels migrating neuroblasts and PCNA (Proliferating Cell Nuclear Antigen), revealed an immunolabelling decrease in SGZ cells in *jnk2*$^{-/-}$ and *jnk3*$^{-/-}$ mice *versus* wt. Moreover, following intraperitoneal injections of kainic acid (KA), an analogue of glutamate, the number of SGZ cells was differentially affected, showing an increase in DCX-immunoreactive cells in *jnk3*$^{-/-}$ treated mice respect untreated *jnk3*$^{-/-}$ mice. All these data suggested that JNK isoforms control the neuronal processes establishment and have specific role in CaBP and the maintenance of adult neurogenesis.

P-4

CHARACTERIZATION OF INFLAMMATORY RESPONSE AFTER MOUSE SPINAL CORD INJURY

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Inflammatory response plays an essential role to protect the body after injury. Inflammation, however, must be a highly regulated response, otherwise, it may lead to tissue damage and/or to chronic inflammation, as observed after spinal cord injury (SCI). Despite the contribution of inflammation to SCI, the recruitment of the different immune cell populations and the expression at the protein levels of cytokines and chemokines in the mouse contused spinal cord has not been fully characterized. Understanding the factors that impede the clearance of immune cells after SCI is likely to be critical for the development of new therapeutic strategies. Herein we assessed the changes in the main inflammatory cell types and cytokine/chemokine expression in the mouse contused spinal cord.

Our results show that neutrophils are the earliest inflammatory cells to invade the injured spinal cord. They peak at 24 hours post-injury and decline progressively up to day 28. Microglial cells peak at day 7 post-injury and slowly decline up to day 28. Microglia, however, is the most abundant immune cell in the contused spinal cord from day 3. The infiltration of monocytes peaks in the contused spinal cord at day 3, and decreases in numbers up to day 14. Few lymphocytes are present within the injured spinal cord for the first week. Strikingly, there is a great influx of lymphocytes into the contused spinal cord at day 21, and their proportion remain elevated up to day 28. The increase of lymphocytes at later stages of spinal cord injury is mainly due to the recruitment of B cells, and to minor degree, to the invasion of CD4 and CD8 T cells.

Regarding cytokine/chemokine expression, our results reveal that all these pro-inflammatory mediators are up-regulated within the first 24 hours, peaking between 6 and 12 hours post-injury. IL6 and G-CSF are the most abundant cytokines at this early phase, and could play a key role in triggering the activation of glial cells and the recruitment of granulocytes. Beyond 24 hours, most of the cytokines were undetectable, except for some of them, such as M-CSF, IL-1α and IL9, that remain elevated until 28 days. Interestingly, the expression of M-CSF peaks at day 3 post-injury, suggesting its importance in monocyte and microglia expansion in the contused spinal cord. Contrary to most cytokines, the expression of chemokines remains at high levels for the first 3-7 days, and some of them, such as CXCL9, CXCL10 and eotaxin, are overexpressed up to day 28.

In conclusion, our data suggest that the lasting up-regulation of some cytokines and chemokines in the injured spinal cord might be responsible, in part, in the failure of immune cell clearance.

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P-5

CSF1R REGULATES MICROGLIOSIS AND DISEASE PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder that affects motor neurons (MNs) in the brain and spinal cord. ALS has a complex pathophysiology involving glutamate-induce cytotoxicity, protein aggregation, cytoskeletal abnormalities and glial cell activation. Although microgliosis is an important hallmark of neurological diseases, the study of microglia in ALS has generated controversial results. In the present study we found that microglia expansion in the spinal cord of $SOD1^{G93A}$ mice correlates with the upregulation of the components of the CSF1 receptor (CSF1R) pathway. In order to evaluate whether CSF1R contributes to microgliosis in this ALS model, we treated $SOD1^{G93A}$ mice with GW2580, a selective CSF1R inhibitor. We found that daily treatment with GW2580, starting at the pre-symptomatic stage of the disease (day 60), reduced microglial cell proliferation in the lumbar spinal cord of $SOD1^{G93A}$. Moreover, inhibition of CSF1R also reduced microglial cell activation but did not interfere with cell polarization. Interestingly, we observed that targeting CSF1R slowed disease progression and increased lifespan of the mice, correlating with an increased preservation of spinal motoneurons. Our findings support that CSF1R plays a crucial role in microgliosis and disease progression in ALS, and suggest that CSF1R inhibitors might be a good therapeutic candidate for the treatment of ALS patients.

P-6

AB IMMUNOTHERAPY REDUCES AMYLOID PLAQUES AND ASTROGLIAL REACTION IN AGED DOMESTIC DOGS

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Background: Alzheimer's disease (AD) is characterized by the dynamic accumulation of extracellular amyloid deposits from the interplay between amyloid-β (Aβ) plaques, reactive astrocytes and activated microglia. Several immunotherapies against Aβ have been shown to reduce amyloid neuropathology. However, the role of the associated glia in the recovery process requires clarification. Previously, we described the safety and effectiveness in aged domestic canine with cognitive dysfunction syndrome of a new active vaccine candidate for the treatment of AD in humans.

Objective: The aim of this article is to gain a better understand of how immunotherapy modifies the amyloid burden and its effects on astroglial and microglial reactivity in immunized dogs.

Methods: In order to achieve this, we compared and quantified amyloid plaques and astroglial and microglial reactions in the frontal cortex of unimmunized and immunized aged domestic dogs.

Results: We found amyloid plaques from immunized dogs to be smaller and more compact than those from unimmunized dogs. In these new plaques, the associated astrocytes were closer and less immunoreactive to S100B. We also found no modification in the microglial reaction associated with immunization.

Conclusion: The anti-Aβ immunotherapy developed in our laboratory modifies the equilibrium between soluble and insoluble Aβ in aged dogs in close correlation with S100B-negative astrocytosis and microglial reaction.

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P-7

M2 PHENOTYPE POLARIZATION OF MICROGLIA AND INFILTRATING MACROPHAGES IN GLIOBLASTOMA MULTIFORME: STUDY IN VITRO AND IN HUMAN BIOPSIES

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Glial tumors or gliomas, are the most common type of primary brain tumors, they present a very poor prognosis, and remain incurable. The presence of an inflammatory environment in gliomas, characterized by glial activation and immune cell infiltration, has suggested that its manipulation could represent a potential therapeutic strategy.
In this study, we aim to elucidate if glioma cells modify the microglial/macrophage-mediated inflammatory immune response in different anatomical compartments within the tumor. On the one hand, by immunocytochemistry, human glioblastoma multiforme (GBM) samples (N=6) were studied in order to correlate the percentage of M1 and M2 tumor-associated microglia/macrophages (TAM/Ms) with the tumors' aggressiveness. In this regard, our findings show that in more aggressive GBMs there is a clear tendency of M2 polarization in the totality of the tissue and in different microenvironments within the GBMs, especially in vascular areas. Moreover, we tried to understand how the nuclear factor-kappaB (NF-κB) pathway is involved in the activation of M1 microglia by means of cell cultures (BV-2 cells). Herein we see that LPS-induced M1 polarization, analyzed by NF-κB nuclear translocation, is not reverted by ibuprofen treatment. Importantly, we observed that when microglia is exposed to both lypopolysaccharide (LPS) and Ibuprofen (Ibu) at the same time, microglial cells appear to overexpress p65 NF-κB, suggesting that M1 phenotype signaling may be potentiated. With this thorough examination of microglial polarization and of the NF-κB intracellular pathway we aim to shed light on how microglia/macrophages could be manipulated in order to suggest therapies for this devastating disease.

P-8

GENE EXPRESSION PROFILING OF LPS- AND LPS+IFNγ-ACTIVATED PRIMARY MURINE MICROGLIA BY RNASEQ

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Microglia, the resident immune cells in the CNS, are thought to participate in the pathogenesis of many neurological disorders. In response to changes in their microenvironment, microglia regulate the level of expression of a number of cellular programs. In particular, microglial cells in the so-called M1 state of activation, upregulate proinflammatory gene expression and they have neurotoxic potential. Our aim has been to analyze the global pattern of gene expression changes induced in microglia in M1 activation. To this end primary cultures of cortical murine microglia were treated with LPS (100 ng/mL) ± IFNγ (1 ng/mL) for 6 hours and RNAs were isolated and analyzed by RNAseq. A significant fraction of the murine genome was expressed by microglia. Thus RNAs encoded by 14302 protein-coding genes, 2178 pseudogenes, 1560 lncRNAs, 168 miRNAs, 306 snoRNAs and 236 snRNAs were detected. Both LPS and LPS+IFNγ induced dramatic changes in microglial gene expression. Significant changes in expression were observed in approximately 30% of protein-coding genes, 12% of pseudogenes, 20% of lncRNAs and 12% of miRNAs. Down-regulations were more frequent than upregulations in protein coding genes and the opposite was true for the other gene classes. Analysis of differentially expressed genes by GO terms revealed significant enrichment in genes involved in immune and inflammatory functions. In summary, these experiments will contribute to our global understanding of the molecular changes involved in microglial activation and to the discovery of novel molecules with functional roles or biomarker properties in neuroinflammation. Supported by La Marato de TV3 (110530) and Instituto de Salud Carlos III, Spain-FEDER funds, European Union (PI10/378 and PI12/709).

P-9

THE MICROGLIAL INHIBITORY RECEPTOR CD200R1 AS A CANDIDATE TARGET TO CONTROL NEUROINFLAMMATION

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Microglia are the main endogenous immune cells of the central nervous system. In response to noxious stimuli, they develop reactive phenotypes to re-establish cerebral homeostasis and minimize neuronal damage. However, reactive microglia produce pro-inflammatory factors with potential neurotoxic effects. Consequently, progress and resolution of microglial activation have to be tightly controlled to avoid negative secondary effects. Neuronal signals play a relevant role in the control of microglial activation. Among them, inhibitory mechanisms such as CD200 ligand (neuronal)-CD200R1 receptor (microglial) interaction, maintain under control the microglia inflammatory phenotype in physiological conditions. Alterations in CD200 and CD200R1 expression have been described in neurodegenerative diseases.

The aim of the present work was to study the effect of CD200R1 modulation on microglia reactive phenotype using experimental *in vitro* approaches. Glial cell cultures were treated with a pro-inflammatory stimulus (LPS+IFN-γ) and the pattern of expression of pro- and anti-inflammatory molecules was determined in the absence and presence of CD200R1 overexpression, CD200R1 stimulation and CD200R1 inhibition. CD200R1 overexpression resulted in a reduced induction of the expression of pro-inflammatory cytokines and enzymes and a strong induction of IL-10 expression in BV2 microglial cells. The induction of the expression of pro-inflammatory molecules was inhibited in reactive primary microglial cells treated with a CD200R1 agonist, while the expression of anti-inflammatory molecules was enhanced. Finally, inhibition of CD200-CD200R1 interaction in mixed glial cultures potentiated the pro-inflammatory response. Thus, the reactive phenotype of glial cells can be modulated through an action on CD200R1 expression or stimulation, suggesting CD200R1 as a candidate target to act against neuroinflammation in neurodegenerative diseases.

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P-10

CD200-CD200R1 SYSTEM IN NEUROLOGICAL DISEASES: MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a complex neurological disease. Although the etiology of MS is unknown, it is widely accepted that it is an inflammatory demyelinating disease of the central nervous system. Microglia play an important role in neurological diseases as executer of innate immunity to restore tissue homeostasis and minimize neuronal damage. However, the exacerbation of microglial activation seems to play a negative role in disease progression, since pro-inflammatory factors overproduction can contribute to neuronal death. Neurons have developed several inhibitory mechanisms to control microglia activation, such as CD200 (mainly neuronal) -CD200R1 (mainly microglial) interaction. Although the neuron-microglia interaction could be a therapeutic target to control glial activation/neuroinflammation, little is known about the mechanisms that regulate this interaction and very few studies are performed in human. In the present work, we studied the expression of CD200-CD200R1 system in an animal experimental model of MS (EAE, experimental autoimmune encephalomyelitis) and in human brain postmortem samples from MS patients. In the EAE model, CD200R1 expression in several spinal cord regions is increased at symptomatic disease phase, while CD200 expression is decreased at both pre-symptomatic and symptomatic phases. In the brain, CD200R1 and CD200 expression are also altered. In MS human samples no significant alterations were observed in mRNA expression for both CD200R1 and CD200. However, CD200R1 protein was increased and CD200 protein was decreased. Therefore, CD200-CD200R1 system may be differentially expressed during development and progression of multiple sclerosis, which suggests that a modulation of CD200-CD200R1 could be a good tool to control microglial activation in this neurological disease.

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P-11

GLYCOGEN-INDUCED NEURODEGENERATION. LAFORA DISEASE

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Glycogen is a branched polymer of glucose that constitutes the sole carbohydrate reserve in mammals. It is synthesized by glycogen synthase (GS), the only mammalian enzyme able to polymerize glucose. Within the brain, glycogen is found mainly in astrocytes, while most neurons do not show detectable levels under physiological conditions. However, in some diseases, glycogen is abnormally accumulated in neurons.

Lafora disease (LD) is a fatal neurodegenerative condition characterized by the accumulation of aberrant glycogen in several cell types, including neurons. LD is caused by mutations affecting two enzymes: malin and laforin. Both enzymes interact functionally to promote the degradation of GS and its activator Protein Targeting to Glycogen (PTG). The causal role of glycogen accumulation in neurodegeneration in LD remains controversial, since the malin-laforin complex may have additional functions to that of the regulation of glycogen synthesis, such as the control of autophagy; in fact, KOs of malin and laforin present autophagy impairment.

To study whether the accumulation of glycogen is primarily responsible for the neurodegeneration in LD we have generated several mouse models with altered capacity to accumulate glycogen in neurons. Our findings reveal that glycogen accumulation is indeed responsible for the neurodegeneration of the malin KO model, as well as for the impaired autophagy. These results identify the regulation of glycogen synthesis as a key target for the treatment of LD and other glycogen-associated neurodegenerative diseases.

P-12

CONTRIBUTION OF MICROTUBULE ALTERATIONS ON AUTOPHAGY AND MOTONEURON CELL DEATH

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Peripheral nerve root avulsion (RA) leads to the interruption of axonal circuitry at the interface between the central and the peripheral nervous systems. This type of lesion produce retrograde motoneuron (MN) degeneration by still unknown mechanisms. It was described that cytoskeletal alterations are key events in this neurodegenerative process as observed in an experimental rat model of RA. Besides, recent proteomic studies pointed to the presence of non-apoptotic executive death pathways involved that occurred simultaneously to some cell-defense pathways such as autophagy. Autophagy was found to be apparently properly unresolved in the *in vivo* model since LAMP-1, a lysosomal protein necessary for the execution of autophagy late-events, was absent within MNs from 5 days post operation (dpo). We confirmed that early alterations on microtubule stability markers and lysosomal enzymes occurred concomitantly in the MNs after RA. In the present study, we aimed to model these simultaneous events, cytoskeletal alterations and autophagy induction *in vitro* to analyze their impact on cell viability. Using the MN-like cell line NSC-34, we observed that microtubule instability early primed cells to die and preclude them to any pro-survival effect triggered by autophagy probably due to alterations in lysosome markers and function.

P-13

MECHANISMS INVOLVED IN RETROGRADE MOTONEURON DEGENERATION AND SURVIVAL

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Traumatic axotomy or mechanical traction (avulsion) of the nerves is caused by accidents that cause irreversible dysfunction and/or monoplegia. In patients who have succeed to perform surgical reconnection of these nerves, only minor recovery of function is achieved in part due to early primary loss of avulsed motoneurons (MNs) by retrograde degeneration. In our experimental model of nerve root avulsion in the adult rat, up to 80 % of MNs are progressive lost through still unknown mechanisms. On the other side, after distal axotomy plus suture of the same nerve, MNs are able to survive and regenerate thereafter. It is not known how MNs can cope with this axotomy stress in that case but not in the former. In order to shed light into this, we have carried out proteomic analysis to compare these models. Besides we generated protein-protein interactomes from 331 protein-seeds that defined 19 motives chosen by manual curation of the literature as main biological processes related with either regenerative or degenerative events in adult spinal MNs. We performed GSEA bioinformatic analysis to confront our proteomic data to these specific interactomes. We validated some results by immunohistochemistry and western blot analysis. Among significant motives those networks associated with autophagic- related events together with those of anti-anoikis are strongly linked to the endogenous neuroprotective mechanisms triggered in MNs to survive in vivo after axotomy. This accurate information can be also a useful platform to develop effective neuroprotective therapies.

P-14

JNK1 AND JNK3 INVOLVEMENT IN FAS SIGNALING PATHWAY INDUCED BY KA

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Epileptic seizures can be induced in rodents by kainic acid (KA), an analogue of glutamate, that replicate many of the phenomenological features of human temporal lobe epilepsy (TLE). KA causes neuronal degeneration in brain, mainly in hippocampus. JNK pathway plays an important role in this neurodegenerative process. There are three genes that encode for three JNK proteins with differential functions depending on cellular context and stimuli. Specifically, JNK3 isoform is mainly expressed in the CNS and has been related to neuronal death. While JNK1 and JNK2 are ubiquitously expressed and seem to be mostly involved in brain development. Different studies have shown that the lack of *Jnk3* confers neuroprotection in neurodegenerative processes.
We examined the potential role of JNK1, JNK2 and JNK3 in neuronal death induced by KA and specifically in apoptotic gene expression levels using a Mouse Apoptosis RT^2 Profiler PCR Array. The results showed a significant reduction of neuronal death induced by KA in *Jnk3* and *Jnk1* Knockout (KO) mice compared to wild-type and *Jnk2* KO mice. The data obtained, after KA treatment, revealed an overexpression of key genes involved in the extrinsic apoptotic pathway, such as *Fasl* and also caspase *Casp3*, *Casp8* and *Casp4,* in wild-type and *Jnk2* KO mice. However these genes were not modified in *Jnk3* and *Jnk1* KO mice.
These findings provide evidence that lacking JNK1 or JNK3 isoforms confers neuroprotection by Fas/FasL extrinsic apoptotic pathway inhibition, suggesting that it could be an important mediator of neuronal apoptosis induced by KA treatment.

P-15

IN SILICO-DESIGNED DRUG COMBINATIONS EXERT NEUROPROTECTIVE EFFECTS IN AN ORGANOTYPIC-BASED CULTURE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Glutamate excitotoxicity and neuroinflammatory reactions are two main features in the neurodegenerative process of Amyotrophic Lateral Sclerosis (ALS). Glutamate re-uptake diminished in synaptosomes from cerebral cortex and spinal cord of ALS patients due to a selective loss of the astroglial transporter in the motor cortex and spinal cord. These changes result in an excessive concentration of glutamate in the synaptic cleft, and the excessive stimulation of glutamate receptors causes an increased intracellular concentration of Na+ and Ca+2, leading to excitotoxic motoneuron (MN) death. On the other hand, the accumulation of reactive microglia is detected in degenerating areas of ALS patients and transgenic mice models. In this work, we assess the potential neuroprotective role in ALS of four compounds identified through a systems biology approach. In particular, we used the Therapeutic Performance Mapping System (TPMS) technology. We validated the therapeutic potential of several drug combinations in cultured spinal cord slices, as they are known to retain the cytoarchitecture of the host tissue and are a well-stablished model to study neuronal death in ALS. Our preliminary results show that combinations of Aliretinoin/Prankulast (C1) and Aliretinoin/Mefloquine (C4) promote neuroprotective effects by reducing the effects of THA chronic excitotoxic insult on both microglia and motoneurons.

P-16

EFFECT OF THE C-TERMINAL DOMAIN OF THE HEAVY-CHAIN OF TETANUS TOXIN ON DYSKINESIA CAUSED BY LEVODOPA IN 6-HYDROXYDOPAMINE-LESIONED RATS

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The carboxyl-terminal domain of the heavy-chain of tetanus toxin (Hc-TeTx) is responsible for the full-toxin´s neurospecific binding, uptake and retroaxonal transport, and activates survivor intracellular signaling in a retrograde manner from distal end-terminals to neuronal soma. The recombinant Hc-TeTx fragment (without toxicity) has been assayed both *in vitro* and *in vivo* as a neuroprotector in Parkinson's disease (PD) models with good success. Until now, levodopa is the gold standard in the therapeutic treatment for PD. The early response to levodopa improves the motor symptoms; unfortunately after some years almost all patients develop motor complications, such as dyskinesia or abnormal involuntary movements (AIMs). We evaluated the co-administration of Hc-TeTx plus levodopa in 6-hydroxydopamine lesioned rats with AIMs expression. The present results suggest that progressive neuronal death causes dyskinesia, which could be prevented by a trophic agent such as Hc-TeTx. The results showed that the animals treated with low levodopa doses (6 mg/kg or 10 mg/kg) did not develop severe dyskinesia, while high levodopa dose (25 mg/kg) treatment produces high AIMs score, and the group with Hc-TeTx plus 25 mg/kg levodopa attenuates limb and orolingual dyskinetic movements. Tyrosine hydroxylase immunoreactivity for midbrain revealed that the Hc-TeTx treatment avoided dopaminergic neuronal death in the tegmental ventral area (VTA) but not in the *Substantia nigra pars compact* (SNpc). In summary, we found that Hc-TeTx could be used in the way to avoid dyskinesias by levodopa, with a lightly protective effect over the dopaminergic neurons.

P-17

SIRT1 OVEREXPRESSION TOGETHER WITH EX527 REDUCE EXCITOTOXICITY-INDUCED NEURONAL LOSS IN CORTICAL NEURONS

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Silent information regulator 1 (SIRT1) is a NAD^+-dependent histone deacetylase that represses gene expression, regulates metabolism, prevents apoptosis and mitigates aging. SIRT1 beneficial effect in neurodegenerative diseases has been widely described, however a protective role in brain ischemia is still questionable. Here, we studied the SIRT1 expression pattern after exposure to NMDA, which receptor is highly activated after ischemia, and the effect of SIRT1 modulation, using lentiviral vectors and pharmacological strategies, on NMDA toxicity in mouse cortical neurons. We observed that SIRT1 levels decline in the cytoplasm, but not in the nucleus from 2h after NMDA addition. Therefore, we transduced neurons with lentiviral vectors that overexpress wt SIRT1 (SIRT1) or a mutant form of the protein with a deletion in the N-terminal sequence (ΔSIRT1). wt SIRT1 that is predominantly expressed in the nucleus and ΔSIRT1 that accumulates in the cytoplasm and the nucleus were not protective against NMDA lesion. However, the combination of increased wt SIRT1 with Ex527, a specific inhibitor of SIRT1 enzymatic activity, reduced neuronal loss. We are currently investigating the mechanisms underlying the protective effect of this treatment. This project is supported by grants from the Fundacio Marato TV3 (110431) and the MINECO (SAF2011-30492).

P-18

NEUROPROTECTIVE POLYPHARMACOLOGY DESIGNED *IN SILICO* FOR MOTOR NEURODEGENERATION AFTER NERVE ROOT AVULSION

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Peripheral nerve root mechanical traction or avulsion due to several kind of accidents or obstetric interventions causes spinal cord damage, dysfunction and monoplegia. The resultant axotomy may lead to a progressive retrograde degeneration of motoneurons (MNs) that finally succumb through still unknown mechanisms and hence thus neuroprotective agents are needed to sustain MN survival. To shed light into these mechanisms, we performed a comparative proteomic study using two experimental rat models: the Root Avulsion model (RA) that produces retrograde degeneration of MNs, and the Distal Axotomy model (DA) that promotes MN survival and regeneration. The resultant data was analysed by a Systems Biology approach and computational methods based on artificial neural intelligence called therapeutic performance mapping system (TPMS). This technology allowed the *in silico* design of several drug pharmacological combinations with putative neuroprotective potential for the RA model. We have explored their neuroprotective potential using both *in vitro* and *in vivo* models. Our results showed that two drug combinations out of three exerted more neuroprotection of MNs than each composite drug alone. Besides, we demonstrated that in silico predicted mechanism of action for at least one combination was correctly deduced. In conclusion, we verified that the present full approach is able to design novel and effective treatments for complex pathological conditions.

P-19

THE CARBOXYL-TERMINAL DOMAIN OF THE HEAVY-CHAIN OF TETANUS TOXIN INDUCES NEURITE OUTGROWTH IN SPINAL CORD MOTORNEURONS

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Tetanus toxin (TeTx), the causative agent of tetanus disease, is a two-chain activated peptide of approximately 150 kDa synthetized by some strains of *Clostridium tetani.* The mature toxin is characterized by three main structural domains: the N-terminal light chain with Zn^{2+}-metalloendopeptidase activity that cleaves the specific SNARE protein synptobrevin/VAMP, and the heavy chain that contains its N-terminal translocation domain of 50 kDa and the C-terminal receptor/binding domain of approximately 50 kDa. The recombinant C-terminal domain of TeTx (Hc-TeTx) has been suggested to act as a neuroprotective and neuroreparative agent by activating signalling pathways related to neurotrophins (e.g.: *Brain-Derived Neurotrophic Factor*, BDNF; *Nerve Growth Factor*, NGF; and *Neurotrophin-3*, NT-3). Hc-TeTx keeps the capacity to be internalised in specialized clathrin-mediated pathway, be retrograde-trafficked to neuronal soma penetrating into central nervous system without toxicity. The objective of the present work is study if Hc-TeTx induce neurite outgrowth in postnatal seven days (P7) rat spinal cord organotypic cultures in a 3D collagen matrix compared with BDNF. Confocal imaging is used to observe neurite outgrowth and then processed with NeuronJ plugin of ImageJ. The results show that Hc-TeTx fragment has de ability to induce the process of neuritogenesis in spinal cord motorneurons and almost mimic the action of BDNF. These findings, and the reported previously by our group that recombinant Hc-TeTx fragment protects spinal motoneurons against excitotoxicity, lead us to propose Hc-TeTx fragment as a new therapeutic agent in front of neurodegenerative diseases and traumatic lesions.

P-20

ROLE OF LIPID RAFTS IN PERIPHERAL NERVE REGENERATION

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Injuries to the peripheral nerves result in loss of motor, sensory and autonomic functions of affected nerves. After peripheral nerve injury different processes begin at distal and proximal sites of injured axons, such as Wallerian degeneration at distal stump and neuronal reaction in the soma of surviving neurons.

In this context, several signals have an important role in the maintenance of the proper degeneration and subsequent regeneration of injured axons. Many of these signals act through membrane receptors that are clustered in discrete microdomains called lipid rafts. Lipid rafts form membrane domains that have a high concentration of cholesterol, sphingolipids and glycolipids, and they cluster different proteins such as neurotrophic factors receptors or protein-kinases. For this reason, lipid rafts are seen as signaling platforms, which can act as a meeting point for signaling molecules.

The aim of this study is to assess the possible role of these microdomains in peripheral nerve regeneration. To this end, lipid rafts were disrupted by administration of methyl-beta-cyclodextrin (MβCD), a cyclic oligosaccharide that removes cholesterol from cell membrane.

Our results in control mice treated with MβCD demonstrate that cholesterol depletion does not affect nerve function and morphology. In mice subjected to a sciatic nerve lesion, electrophysiological tests indicate that lipid raft deconstruction by MβCD accelerates muscle reinnervation after axotomy. These results are also supported by findings of increased responses of sensory reinnervation.

Therefore, we conclude that lipid raft disruption may accelerate nerve regeneration after axotomy by promoting a better microenvironment for axonal growth.

P-21

FUNCTIONAL RECOVERY MEDIATED BY ENRICHED ENVIRONMENT AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS

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Objectives: Enriched environment (EE) promotes functional recovery in animal models of stroke. Here, we used fMRI to assess temporal dynamics of brain recovery after stroke in rats, comparing different housing conditions.

Methods: Transient brain ischemia (90 min) was induced in adult male Wistar rats. Groups of sham-operated rats were carried out in parallel for both housing conditions. Three weeks after stroke, animals were randomly distributed in either EE (n=11) or STD (n=12). EE consisted in large cages, with 2 levels, ramps, a running wheel, wooden blocks, among other items that were modified 2 times per week. Rats were studied for 12 weeks.

MRI: T2 maps (7T, Bruker) were acquired to measure the lesion size. fMRI was performed every three weeks. Electrical forepaw stimulation was repeated 5 times (15sec each) with resting state periods in between (45sec each). Statistical activation maps were constructed with the software STIMULATE.

Behavioral test: Cylinder test for asymmetries and Rotarod test for motor coordination were performed the day before each MRI session.

Results None of the rats kept under STD showed signs of behavioural recovery, while EE significantly improved motor coordination and ameliorated motor asymmetries in relation to STD. In fMRI, 42% of the EE rats showed activity recovery (BOLD) between week 9 and 12, whereas this effect was not observed in any of the STD rats.

Conclusion fMRI provides useful information of activated areas after forepaw stimulation. EE promotes the functional recovery of the original representation field, as assessed with fMRI and behavioural testing.

P-22

EFFECT OF UTP IN SCHWANN CELL PHENOTYPE DURING NERVE REGENERATION.

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Nucleo CMP forte (CMPF) is a drug currently used as a supplementary treatment in patients with neuropathic pain of different origins. CMPF is composed of cytidine monophosphate (CMP), uridine monophosphate (UMP), uridine diphosphate (UDP) and uridine triphosphate (UTP).

After injury in a peripheral nerve, several molecules, such as nucleotides, are released from the injured cells, leading to several molecular and cellular changes in order to repair the damaged nerve. In Schwann cells, these changes involve dedifferentiation, migration to the injured area and finally, redifferentiation and contact with the regenerating axons.

Our research group has demonstrated that UTP is the most effective component of CMPF in our *in vitro* assays, showing that it is able to induce schwannoma cell migration through activation of P2Y2 receptors and through the increase of extracellular matrix metalloproteinases.

In this work, we have studied the potential role of UTP at different stages of nerve repair, in particular, differentiation, dedifferentiation and migration of Schwann cells. To this purpose, we have treated primary Schwann cell cultures with UTP and we have studied the expression of specific markers of differentiation and dedifferentiation. For the migration assay, we have used the boyden chamber system.

We have found that UTP treatment induce migration in Schwann cell, and keeps the dedifferentiation phenotype. Our results point to a potential role or UTP during the early stages of nerve repair.

P-23

SUSTAINED RELEASE OF NEUROTROPHIC FACTORS WITH PLGA MICROSPHERES IMPROVES NERVE REGENERATION

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After nerve transection, axonal regeneration and functional recovery are poor when axons have to regenerate through long distances. Although nerve autograft is still considered the gold standard in peripheral nerve regeneration, procedure invasiveness and limited nerve availability is a limitation to this approach. Nerve guide conduits (NGCs) filled with extracellular matrix and neurotrophic factors (NFs) have been widely used to repair transected nerves. If the NF can be delivered locally along time and not in an initial burst, axonal regeneration should be enhanced.

Aims: To examine the effect of different NFs, free or encapsulated in PLGA microspheres, on axonal regeneration after nerve injury.

Methods: NGC filled with a collagen matrix and free or encapsulated NFs were used to repair a 6 mm gap resection of the rat sciatic nerve. Groups were divided as free or encapsulated NGF, NT-3, GDNF, FGF-2 and controls without NF. Twenty days after repair, Fluorogold was applied and spinal cord and DRG (L4 and L5) labeled neurons were quantified.

Results: In comparison with free NFs, encapsulated NGF, NT-3 and GDNF increased the number of regenerated motoneurons, while NT-3, GDNF and FGF increased the number of regenerated DRG neurons. Furthermore, all the encapsulated NFs as well as free GDNF increased the regenerated motoneurons compared to control groups. Moreover, free and encapsulated NGF and encapsulated GDNF increased DRG regenerated neurons.

Conclusion: NF encapsulation in microspheres enhances axonal regeneration in comparison with free NFs when applied within nerve conduits in vivo.

P-24

SCIATIC NERVE AVULSION VERSUS L4-L5 ROOT AVULSION LEAD TO TOTALLY DIFFERENT DEGENERATIVE OUTCOMES

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Road traffic accidents are the most common cause of peripheral nerve root avulsion injury producing a disconnection between spinal cord and spinal roots. Several rat models have been proposed to study the mechanisms of retrograde degeneration account to motoneurons (MNs) after this kind of injury. In the present study, we compare two rat models of injury: one caused by sciatic nerve avulsion (SA) and the other by L4-L5 root avulsion (RA). Spinal nerve-avulsioned MNs showed increased levels of cleaved pro-apoptotic caspase-3, nuclear localization of the AIF pro-apoptotic protein and chromatin compactation by 14 days after surgery. In contrast, these apoptotic features cannot be detected after L4-L5 root avulsion suggesting that another unknown death mechanism might be involved. Ultrastructurally, there were notable differences since MNs after RA usually showed nuclear and cell body enlargement while in SA-injured MNs there were chromatin marginalization, a typical apoptotic feature, instead. This apoptotic features were not detected in MNs from the RA model. This study highlights that experimental subtilites can lead to totally different molecular mechanisms regarding degeneration that are relevant when considering therapeutic options. Besides, our nerve root avulsion model provides an excellent platform to investigate new undiscovered mechanisms of motoneuron degeneration.

P-25

HERICIUM ERINACEUS EXTRACT IS A NEUROIMMUNOMODULATORY AND NEUROPROTECTIVE TREATMENT AGAINST NMDA-INDUCED EXCITOTOXIC LESION

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Background: *Hericium erinaceus* is a mushroom that has immunomodulatory properties and neuroprotective effects, inducing overexpression of NGF in certain brain regions. Microglial cells have a fundamental role in neuroprotection and neurodegeneration and their activation is one of the main cellular events in neuroinflammation in CNS.

Aims: We investigated the neuroprotective effects of a treatment with *H. erinaceus* extract (HEE) and its potential molecular mechanism in the rat model of NMDA induced hippocampal neurodegeneration. We also investigated the effects of erinacine A and HEE fractions in primary microglial cultures and BV2 cells.

Methods: Animals were orally treated with HEE during 14 days after NMDA injury. Hippocampal regions were analyzed by immunohistochemistry. Gene expression of the hippocampus was assessed using Illumina beadarrays. We performed a dose-response *in vitro* study of the effect of HEE, and its extracted fractions, on BV2 cells and primary microglial cultures. Inflammatory status was assessed by Nitric Oxide (NO) production.

Results: *In vivo,* the HEE treated animals exhibited a 58% reduction of the hippocampal lesion volume and the microglial reaction. The gene expression analysis indicated that HEE treatment produces an enriched pattern of genes related with the immune system, MHCII processing and presentation, suggesting the involvement of microglial cells. *In vitro*, HEE organic fraction at high concentration, and Erinacine A, significantly decrease the production of NO in activated primary microglial cultures and BV2 cells.

Conclusions: Our results point towards HEE being a neuroimmunomodulatory treatment useful for preventing or delaying the progression of neurodegeneration of an excitotoxic lesion.

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P-26

HIPERALGÈSIA TÈRMICA I EXPRESSIÓ DE RHO-A EN RATOLINS TRACTATS AMB IBUPROFÉ I POLIFENOL DESPRÉS DE CONTUSIÓ DE LA MEDUL·LA ESPINAL

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L'Ibuprofé és un fàrmac anti-inflamatori no esteroïdal (AINE) utilitzat per reduir el dolor i la inflamació via inhibició de les ciclooxigenases. L'Ibuprofé també inhibeix la via intracel·lular de senyalització de Rho-A, fet que pot afavorir el creixement axonal, i la recuperació funcional després de lesions de la medul·la espinal. L'Ibuprofé disminueix el dolor neuropàtic postlesió medul·lar, però més controvertits són els estudis sobre promoció de la recuperació motora i la regeneració axonal central. En aquest estudi s'ha avaluat l'efecte del tractament amb ibuprofé (IBU) i amb (-)-epigalocatequin galat (EGCG) en el temps de retirada a un estímul tèrmic dolorós (algesimetria tèrmica), i en l'expressió de Rho-A en mostres de medul·la espinal processades per western-blot, de ratolins sotmesos a una contusió de la medul·la espinal (T8-T9) realitzada mitjançant la tècnica de la caiguda de pes (2 g de pes a 25 mm d'alçada). Els animals control van ser tractats amb els vehicles dels dos fàrmacs. A 7 i 14 dies postoperació (dpo), el temps de retirada dels animals tractats amb IBU i EGCG van ser superiors als dels animals control, però només amb diferències significatives en el grup EGCG respecte al control. Amb els dos tractaments s'observa una tendència a disminuir l'expressió de Rho-A respecte als animals tractats amb vehicle. Aquest resultats suggereixen que els tractaments amb ibuprofé i EGCG redueixen el dolor neuropàtic central mitjançant modulació de l'expressió de Rho-A.

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P-27

LA INTOXICACIÓ CRÒNICA PER 3,3'-IMINODIPROPIONITRIL CAUSA HIPERALGÈSIA TÈRMICA ASSOCIADA A ALTERACIONS A LA BANYA DORSAL DE LA MEDUL·LA ESPINAL DE LA RATA

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El 3,3'-iminodipropionitril (IDPN) és un agent tòxic que provoca axonopatia per acumulació de neurofilaments en els segments proximals dels axons. La intoxicació amb IDPN causa alteracions motores i sensorials associades als sentits especials. L'objectiu d'aquest treball ha estat estudiar la resposta nociceptiva mitjançant algesimetria tèrmica plantar, en rates tractades amb IDPN (15 mM en aigua de beguda) durant 5 setmanes, i avaluar per tècniques d'immunohistoquímica els canvis en la gliosi, densitat de fibres nociceptives aferents i neurones positives a calbindina-D28k a la banya dorsal de la medul·la espinal. Els animals control només van rebre aigua de beguda. Els resultats mostren que a les 5 setmanes d'exposició els animals tractats amb IDPN presenten un temps de retirada a l'estímul dolorós tèrmic significativament inferior als animals control. El grau d'immunoreactivitat a GFAP (astròcits), IBA-1 (micròglia) i a CGRP (fibres aferents C peptidèrgiques) no va mostrar canvis significatius entre els animals control i els tractats amb IDPN a les 5 setmanes d'exposició. Per contra, els recomptes de cèl·lules positives per calbindina-D28k i el de cèl·lules marcades amb isolectina B4 (fibres aferents C no peptidèrgiques) a la banya dorsal van augmentar en animals tractats amb IDPN respecte als controls a les 5 setmanes d'exposició. Aquests resultats suggereixen que la intoxicació per IDPN causa dolor neuropàtic associat a canvis de les projeccions nociceptives no peptidèrgiques i d'interneurones positives a calbindina a la banya dorsal, sense causar gliosi.

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P-28

EL TRACTAMENT AGUT AMB EPIGALAT REDUEIX LA HIPERALGÈSIA TÈRMICA I L'EXPRESSIÓ DE FRACTALQUINA DESPRÉS DE CONSTRICCIÓ CRÒNICA DEL NERVI CIÀTIC EN EL RATOLÍ

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(-)-Epigallocatequin galat (epigalat o EGCG) és el polifenol més abundant en el té verd. En models experimentals de dolor neuropàtic, el tractament amb EGCG disminueix la hiperalgesia i l'al·lodinia. El dolor neuropàtic per lesió de nervi causa l'alliberament de fractalquina en la banya dorsal de la medul·la espinal, quimocina responsable de la reactivació glial. L'activació glial amb alliberament de citoquines proinflamatòries és la causa principal de generació i manteniment del dolor neuropàtic postlesió nerviosa. En aquest estudi s'ha avaluat la hiperalgèsia tèrmica mitjançant algesimetria tèrmica plantar en ratolins sotmesos a constricció crònica del nervi ciàtic i que han estat tractats amb EGCG (50 mg/kg; i.p.; diàriament durant 7 dies). Els animals control van ser tractats amb vehicle (10% DMSO en solució salina estèril). Als 14 dies postlesió, es van extreure les medul·les espinals lumbosacres, que van ser processades per western-blot en la determinació dels nivells de fractalquina (CX3CL1). Els resultats mostren que els animals tractats amb EGCG presenten un temps de retirada a l'estímul tèrmic dolorós significativament superior als animals control, i que l'expressió de fractalquina és lleugerament inferior en els animals tractats amb EGCG respecte als controls. Aquests resultats suggereixen que el tractament agut amb EGCG redueix el dolor neuropàtic mitjançant modulació de l'expressió de fractalquina.

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ACID-SENSING ION CHANNELS CONTRIBUTION TO OCULAR PAIN.

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Sensory nerve terminals in the eye anterior surface detect external stimuli producing innocuous and painful sensations. Ion channels present in nerve terminals play a key role in sensory detection and their expression and properties undergo profound changes in conditions such as dry eye, chronic inflammation or ocular surface lesions. Sensory neurons in the ocular anterior surface are subject to significant pH changes during inflammation or tissue injury and protons are among the first mediators released by damaged cells. We studied whether Acid-Sensing Ion Channels (ASICs) are expressed in corneal sensory neurons as well as their role in response to moderate acidifications of the ocular surface and in pathologies that produce ocular surface inflammation. Moderate acidic pH (6.6) activated ASIC-like currents in corneal polymodal sensory neurons, which were blocked by ASIC1 or ASIC3-specific toxins. Acidic pH depolarizes corneal sensory neurons to fire action potentials, an effect blocked by APETx2, an ASIC3 blocker. Moderate acid stimulus produced nocifensive behaviors (blinking, scratching) that were prevented by ASIC blockers. Ophthalmic drugs formulated in an acidic solution produced similar effects. In a model of allergic keratoconjunctivitis, nocifensive behavior was greatly reduced by ASIC3 blockade, likely reducing nociceptor sensitization during the inflammatory process. These results show that ASICs, together with TRPV1, play a significant role in the detection of acidic insults in the ocular surface. The identification of ASIC channels in corneal neurons as well as their alterations in different ocular pathologies are critical for understanding sensory physiology and may represent novel targets for the development of new therapeutic agents for ocular pathologies.

P-30

ROLE OF TRESK POTASSIUM CHANNELS ON ITCH AND PAIN

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TWIK-RElated Spinal cord K^+ channel (TRESK) is a member of the two-pore domain potassium channel (K_2P) family and is highly expressed in dorsal root ganglion and trigeminal sensory neurons. TRESK is a major contributor to background K^+ currents and is crucial for maintenance of resting membrane potential and neuronal excitability, helping to prevent excessive sensory neuron activation. Injury of sensory neuron axons, inflammation, migraine and altered sensory perception has implicated TRESK in nociception and mechanotransduction, making this channel a potential therapeutic target for pain-related disorders. To determine the role of TRESK in sensory transduction we studied its sensitivity to changes in membrane tension (stretch) by inducing laminar stress to the cells, producing cell swelling and shrinkage or treating the cells with membrane crenators or cup-formers. We also tested the potential modulatory effect of imiquimod (IQ) on TRESK, an itch-inducing compound known to depolarize sensory neurons; and deltamethrin (DM) or tetramethrin (TM), insecticides that induce sensory alterations in humans such as paraesthesias and burning and stinging painful sensations. Our results provide evidence that TRESK is modulated by changes in cell membrane tension and curvature. During inflammation, hypertonic conditions together with inflammatory mediators like arachidonic acid and acidic pH have additive effects inhibiting the channel to promote nociceptor activation. Inhibitory effects of imiquimod or pyrethroid insecticides on TRESK are likely to mediate neuronal activation to produce the itching and painful sensations reported by patients.

P-31

DEGENERACIÓ I REPARACIÓ AFERENT VESTIBULAR EN UN MODEL EXPERIMENTAL D'OTOTOXICITAT PER 3,3'-IMINODIPROPIONITRIL EN RATOLÍ

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El 3,3'-iminodipropionitril (IDPN) és un nitril neurotòxic que provoca disfunció vestibular. En rates, la intoxicació crònica per IDPN causa retracció de les fibres aferents que innerven les cèl·lules ciliades vestibulars. Aquesta retracció aferent precedeix a la pèrdua de cèl·lules ciliades i a la disfunció vestibular. La recuperació funcional està associada a la reparació aferent. L'objectiu d'aquest estudi és desenvolupar un model animal per estudiar el dany i la reparació de les fibres aferents que es produeix en la intoxicació crònica per IDPN en ratolí. Ratolins de la soca 129S1 i Swiss van ser intoxicats amb IDPN a diverses concentracions (20, 25, 30, 35, 40 mM) en aigua de la beguda durant 4 o 5 setmanes. Posteriorment es va interrompre l'exposició d'IDPN i es va administrar aigua sense IDPN durant 5 setmanes més *(*període *washout).* Setmanalment es va determinar el pes corporal i es va avaluar la funció vestibular. A les 4-5 setmanes d'exposició d'IDPN i al final del seguiment (5 setmanes de *washout*), es van recollir mostres d'epitelis vestibulars que van ser processades per tècniques d'immunocitoquímica per visualitzar tenascina-C, Caspr, KCNQ4, MyoVIIA i NF200. Els resultats mostren que la concentració òptima per a estudiar els processos moleculars i funcionals associats a la degeneració i reparació eferent vestibular per intoxicació crònica d'IDPN és de 30mM en ratolins mascle 129S1. Aquesta concentració no és efectiva en ratolins Swiss mascle i causa excessiva toxicitat sistèmica en els 129S1 femella.

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INVESTIGATING THE TRAFFICKING MECHANISMS OF TRESK, A TWO-PORE DOMAIN POTASSIUM CHANNEL IMPLICATED IN PAIN

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The K2P channel TRESK (K2P18.1) has been implicated in pain perception pathways and changes in channel expression and function have been reported to alter the excitability of sensory neurons. There is a growing awareness of the importance of TRESK as a therapeutic target with potential use in the treatment of a host of pain-related disorders, including migraine.

TRESK K^+ currents can be modulated by several distinct factors including extracellular pH, arachidonic acid, phosphorylation, membrane tension and shear stress. Despite the importance for the overall current density, there is almost no information regarding the mechanisms by which the total number of TRESK channels at the cell surface are controlled.

To investigate factors affecting TRESK trafficking to and from the cell surface we have developed biochemical (surface biotinylation) and surface immunofluorescence assays which we use in conjunction with electrophysiological analysis. Our work is focused on two main questions: (i) What are the determinants of trafficking in the TRESK protein? (ii) How is TRESK trafficking affected under pathophysiologal conditions, for example, during inflammation?

We are currently analysing the role of a tyrosine-based endocytic motif in the short C-terminus of TRESK and the effect of PMA, an activator of Protein Kinase C, on the surface expression of rat TRESK expressed in HEK293 cells. Our initial results indicate that surface expression of TRESK does not appear to be affected by mutation of the C-terminal endocytic motif. In contrast, PMA treatment appears to increase surface expression of the channel and whole-cell TRESK K^+ currents.

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ESTUDI FUNCIONAL I IMMUNOHISTOQUÍMIC DEL DOLOR NEUROPÀTIC EN LA LESIÓ FOTOQUÍMICA DE LA MEDUL·LA ESPINAL DEL RATOLÍ

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La lesió fotoquímica de la medul·la espinal, mitjançant l'aplicació d'un agent fotosensible i posterior il·luminació amb llum visible, és un model experimental utilitzat en rata per estudiar aspectes fisiopatològics de la lesió medul·lar, i mecanismes de reparació i regeneració central mitjançant teràpies fàrmacocelul·lars. En aquest estudi s'han utilitzat ratolins femelles Balb-c que van ser sotmesos a laminectomia dorsal (T8-T9), bany de la medul·la espinal exposada amb 1,5% de rosa de bengala (RB) i il·luminació amb llum freda durant 1, 2, 5 i 10 minuts. Els animals control no van ser il·luminats. Durant les primeres 8 setmanes postoperació, setmanalment s'ha avaluat la locomoció en camp obert mitjançant escala BMS, i temps de retirada a estímul tèrmic dolorós mitjançant algesimetria tèrmica plantar. Al final del seguiment els animals van ser perfosos i extretes les medul·les espinals de l'àrea de lesió, que van ser processades per tècniques d'inmmunohistoquímica per visualitzar astròcits (GFAP),i fibres aferents nociceptives peptidèrgiques (CGRP) de la banya dorsal. El resultats funcionals mostren que a major temps d'il·luminació menor recuperació motora en l'escala BMS, i menor temps de retirada a l'estímul dolorós. La immunoreactivitat a GFAP i CGRP augmenten amb el temps d'il·luminació. Aquests resultats suggereixen que en el ratolí la lesió fotoquímica causa dolor neuropàtic central associat a un augment de la gliosi i de la densitat de fibres nociceptives aferents en la banya dorsal.

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$STEP_{61}$ LEVELS MODULATE NOCICEPTION: EFFECT OF AGE AND GENDER

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Nociception is the process by which primary sensory neurons detect pain-producing stimuli. The information from the nociceptors is processed in the dorsal horn of the spinal cord by complex circuits involving excitatory and inhibitory interneurons. Glutamate receptors and extracellular signal-regulated kinases 1/2 (ERK1/2) play an important role in signaling during nociception. STriatal-Enriched protein tyrosine Phosphatase (STEP) is involved in neuronal signal transduction through the dephosphorylation of important signaling molecules, including the N-methyl-D-aspartate (NMDA) glutamate receptor subunit GluN2B and ERK2. Its mRNA is alternatively spliced into two major isoforms, the cytosolic $STEP_{46}$ and membrane-associated $STEP_{61}$. In the present work we aimed at determining the contribution of STEP to nociception by using STEP knockout mice and TC-2153, a recently synthesized pharmacological inhibitor of STEP. Only the $STEP_{61}$ isoform was detected in mouse lumbar spinal cord extracts. Our results showed that genetic and pharmacological inhibition of STEP produces thermal and mechanical hyperalgesia in mice, as assessed by the Hargreaves and Von Frey tests. At the molecular level this inhibition correlates with increased levels of $pGluN2B^{Tyr1472}$ and $pERK2^{Tyr204}$ in the lumbar spinal cord. Importantly, electrophysiological experiments showed that TC-2153 increases C fiber-evoked spinal field potentials in rats. Moreover, we found that $STEP_{61}$ protein levels in the lumbar spinal cord inversely correlate with the increased thermal hyperalgesia associated with age and female gender. Collectively, these results support a role for spinal STEP levels in the modulation of nociception, and highlight a potentially relevant target for pain management.

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AFFERENT PATHOLOGY AND REPAIR ASSOCIATE WITH VESTIBULAR DYSFUNCTION AND RECOVERY DURING CHRONIC OTOTOXICITY IN THE RAT

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We aimed at identifying key pathological events associated with loss of vestibular function during chronic ototoxic exposure. Adult male Long-Evans rats were exposed to 10 and 20mM of 3,3'-iminodipropionitrile (IDPN) in the drinking water for 4 or 10 weeks. The animals were studied at the end of the exposure, or after 4 or 10 weeks of recovery. Our protocol allows comparing behavioral data of vestibular dysfunction with ultrastructural and immunochemical data on an individual basis. After 4 weeks of 20mM most animals showed significant vestibular impairment. SEM and TEM observations showed intact overall epithelial structure, with no evidence of stereocilia coalescence or hair cell (HC) degeneration or extrusion. The electron-dense material characterizing the septate junction between afferents and HCIs was no longer present in most calyx endings; some terminals had retracted and only partially covered the HCI membrane, but the afferents were not swollen. Confocal microscopy data showed a dramatic loss of the septate junction protein caspr. Animals exposed to 10mM showed little or no behavioural or epithelial changes. In the more severely affected animals after 4 weeks of 20mM, and in animals exposed for longer times, coalescence of stereocilia and HC extrusion were observed. Comparison of animals with similar behavioural dysfunction examined right after exposure or after a recovery period indicated that behavioural recovery associates with afferent repair. We conclude that loss of septate junctions, synaptic uncoupling, and afferent retraction are reversible events associated with the initial loss of vestibular function during chronic ototoxic exposure.

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PHOSPHATASE DUSP6 REGULATES HIPPOCAMPAL ERK1/2 ACTIVATION AND CONTEXTUAL MEMORY

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Transient activation of the Erk/MAP kinase pathway in the hippocampus is essential for memory consolidation and long-term neuroplasticity. Although negative regulation of Erk1/2 may be just as important for memory as stimulatory mechanisms, little is known about the hippocampal phosphatase(s) that directly inactivate Erk1/2 during memory formation. Here we show that Dusp6 (a dual-specificity Y/T-phosphatase) is a neuron-specific cytosolic protein enriched in pyramidal dendrites of the hippocampus. Notably, binding between Dusp6 and Erk1/2 increased during memory formation, coinciding with the trough of Erk1/2 phosphorylation. The allosteric Dusp1/6 inhibitor, BCI, triggered a cell-wide increase in Erk1/2 activation in cultured neurons of wild type (WT) and Dusp6 knockout (KO) mice, suggesting that BCI stimulates Erk1/2 signaling through targets other than Dusp6. We then used KO mice to study the role of Dusp6 in memory formation. KO mice showed normal body growth and brain anatomy. Basal behavior, motor coordination and anxiety were similar between WT and KO mice. In contrast, KO mice had severe deficits in contextual fear memory. Collectively, the results indicate that Dusp6 dynamically interacts with Erk1/2 to regulate the duration of learning-induced MAPK signaling in the hippocampus, and identify Dusp6 as a novel candidate regulator of memory formation.

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REGIONAL BRAIN NEUROTRANSMITTER LEVELS: SEX, HALOTHANE GENOTYPE AND COGNITIVE BIAS.

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The development of methods for assessing emotional states is a crucial step in improving animal welfare. The "cognitive bias", defined as a pattern of deviation in judgment in particular situations, is used as a label for the effects of affective state on cognitive processes.

The aim of this study was to determine the concentration of indoleamines (5-hydroxyindole-3-acetic acid (5-HIAA) and serotonin (5-HT)) and catecholamines (noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)) in the amygdala, hippocampus and prefrontal cortex of a group of slaughtered pigs classified according their emotional state, sex and halothane genotype.

The study was carried out on 48 hybrids Large White-Landrace pigs housed at *IRTA-Monells* facilities. Animals were trained to learn to discriminate positive and negative spatial cues and classified according to their emotional state during the cognitive bias test.

Regional distribution of brain monoamines showed similar patterns to those described in the literature and our previous results (UFAW-Meeting, 2013).

Between sexes, halothane-carrier-males showed lower concentration of dopamine and its metabolites in the amygdala, whereas halothane-carrier-females showed higher concentrations of serotonin and its metabolites in the hippocampus.

When considering the effect of cognitive bias, females defined in a optimistic state showed higher concentrations of homovanillic acid ($p<0.05$) in the amygdala, probably related to the tendency to decrease in dopamine concentration, suggesting a relationship between motivation and cognition.

We conclude that halothane genotype produces changes in neurotransmitter's concentration of slaughtered pigs. Furthermore, we also found differences in dopamine metabolism in the amygdala between female pigs defined as optimistic or pessimistic.

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PREFRONTAL BRAIN ACTIVATION IN INHIBITED SUBJECTS IN RESPONSE TO VIEWING PICTURES WITH DIFFERENT EMOTIONAL VALENCE

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This study investigated the prefrontal processing underlying the cognitive control of emotions induced by pictures with different emotional load in subjects with highest values of behavioural inhibition. Previous studies have suggested that left and right sides of Prefrontal Cortex (PFC) are engaged in different ways of emotional control processes. The frontal hemodynamic response that occurred in the prefrontal region was measured in a quiet room using a 16-channel functional near-infrared system (fNIR100A, BIOPAC Systems, Inc., USA). Twenty slides were selected from the International Affective Picture System (IAPS), 10 pleasant and 10 unpleasant pictures. Subjects were 12 females (mean 19.75±1.54 years). There was a difference in the oxygenation scores for pleasant and unpleasant conditions at voxel 2 ($t(9) = 3.04$, $p = 0.01$), voxel 4 ($t(9) = 3.19$, $p = 0.01$), and a tendency towards the significance at voxel 1 ($t(11) = 1.94$, $p = 0.08$) in the right hemisphere. There was also significant differences in the left hemisphere at voxel 11 ($t(8) = 2.55$, $p = 0.03$) and a tendency towards the significance at voxel 15 ($t(11) = 1.87$, $p = 0.09$) and at voxel 16 ($t(9) = 2.16$, $p = 0.06$). Pleasant pictures increased oxygenation level whereas unpleasant pictures decreased it. Our study is in accordance with previous fNIRs studies showing prefrontal activation during emotional processing.

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LONG-LASTING WITHDRAWAL OF CHRONIC COCAINE SELF-ADMINISTRATION REGULATES CREB PHOSPHORYLATION IN THE NUCLEUS ACCUMBENS SHELL

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Cocaine abuse leads to long-lasting neuroadaptations in brain reward circuits. The role of the transcription factor cyclic-AMP response element binding protein (CREB) in addiction is far from clear, but it may contribute to mood regulation and to the motivation for cocaine reinforcement among other functions.

We assessed the enduring changes in phosphorylation of CREB in fresh brains obtained from rats after 6 to 13 weeks of daily i.v. cocaine self-administration plus one day or 5 weeks of withdrawal. Slices from these or control animals animals were incubated *in vitro* with or without the dopamine D_2 receptor agonist quinpirole before slicing for pCREB-like immunohistochemistry.

In control rats and slices, the shell of the nucleus accumbens showed higher density of pCREB positive nuclei than other striatal regions. The number of pCREB-positive neurons in the nucleus accumbens shell decreased both one day and 5 weeks after discontinuation of cocaine self-administration as compared with control rats. Incubation of the slices with the dopamine D_2 receptor agonist quinpirole elicited opposite effects in brains obtained from control and cocaine-treated animals.

These findings partly support a previous report from our group using western blot where quinpirole strongly increased pCREB levels in the nucleus accumbens after cocaine self-administration [1]. A long cocaine withdrawal maintains low levels of pCREB positive nuclei in the nucleus accumbens shell, and dopamine D_2 stimulation with quinpirole tends to normalize such decrease. Our findings suggest that pCREB could be involved in maintaining the long-lasting changes that characterize memory of cocaine experience.

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EFFECT OF HIGH-FAT DIET AND RESVERATROL ON SAMP8 COGNITIVE DEFICITS AND BEHAVIOR

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The causes of aging remain unknown, but probably they are related to a multifactorial process. To date, the free radical and mitochondrial theories seem to be the two most prominent theories of aging and have survived the test of time. Besides is known that increased adiposity induced by a high-fat diet accelerates the aging process and aggravates the amyloid and tau pathology in mouse models. If aging associated processes result from oxidative stress, it may be corrected by antioxidants like resveratrol. Resveratrol, a natural polyphenolic compound that is found in grapes and red wine, increases metabolic rate, insulin sensitivity, mitochondrial biogenesis, and physical endurance and reduces fat accumulation in mice.

This work has principal aim the study of the effect of high-fat diet in SAMP8 and if these diets accelerate the appearance of cognitive impairment and secondly whether resveratrol is able to reverse effects caused by the high-fat diet. We found that administration of high-fat in SAMP8 after weaning until 4 month of age increased cognitive impairment in SAMP8 mice but not significantly in reference to control diet fed SAMP8. However, the resveratrol reversed the cognitive impairment caused by high-fat diet in a significant way. Furthermore high-fat diet modified the emotional and social behavior in SAMP8 at 3-4 months of age, nevertheless no significant changes were observed with resveratrol.

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DIFFERENT MECHANISMS DRIVE ACTIVATION OF ERK/MAP KINASE DURING MEMORY CONSOLIDATION AND RETRIEVAL IN THE SAME CA1 NEURONS

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Increases in CREB-dependent transcription in CA1 pyramidal neurons of the hippocampus are critical for the consolidation of episodic memories. Prior studies indicated that NMDAR-dependent activation of Erk1/2 supports CREB-mediated transcription and memory formation. However, the role and mechanisms of activation of Erk1/2 during memory recall are far less understood. In mice trained for contextual fear memory, context re-exposure triggered a major increase in Erk1/2 activation in CA1 pyramidal neurons. In contrast, context re-exposure in the absence of memory recall did not activate Erk1/2. Further, local blockade of Erk1/2 shortly before re-exposure suppressed retrieval of the conditioned fear. Analysis of the overlap between neuronal ensembles showing CREB-dependent transcription during learning and those showing Erk1/2 activation during retrieval indicated that Erk1/2 is preferentially reactivated in the same subset of CA1 neurons. Intriguingly, NMDAR antagonists blocked long-term memory formation but spared memory recall. In keeping with this, NMDAR antagonists blocked Erk1/2 activation in CA1 neurons during training, but not during memory retrieval. Therefore, Erk1/2 required for memory consolidation and retrieval is largely reactivated in the same neuronal ensembles albeit through distinct molecular mechanisms.

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A CELL MODEL FOR RETT SYNDROME SUITABLE FOR CHARACTERIZATION OF CELLULAR ALTERATIONS AND PRECLINICAL STUDIES

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Introduction: Rett syndrome is a neurodevelopmental disorder of low prevalence (1:10000), occurring almost always in girls, and mainly caused by mutations in the methl-CpG-binding protein 2 (MeCP2). This gene is located in the X chromosome and codes for a nuclear protein involved in chromatin regulation, gene silencing and transcription regulation.

Aims: development of a cell model suitable for the study of Rett syndrome; characterization of the biochemical properties and cell biology of two mutations of MeCP2 causing Rett (R255X and R306C).

Materials: a commercially available ShRNA to eliminate endogenous protein and specifically designed constructs of MeCP2 are stably introduced into the human cell lines 293T and SHSY5Y using a lentiviral vector delivery system. Protein biochemistry and cellular properties are studied by western-blot and immunofluorescence.

Results: we report a global and stable knocking down of endogenous MeCP2 and high and stable expression of MeCP2 wild-type, R255X and R306C with no remarkable interference of the ShRNA. The effect of the two mentioned mutations on protein levels and subcellular distributions is also shown.

Conclusions: we have developed a cellular model suitable for the study of mutations of MeCP2 involved in Rett syndrome and report the effect of the mutations R255X and R306C in protein stability and subcellular distribution. Potential use of this model for characterization of alterations at the cellular level caused by mutations of MeCP2 and preclinical studies of new treatments is also discussed.

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SELECTIVE KNOCKDOWN OF TASK3 POTASSIUM CHANNELS IN MONOAMINERGIC NEURONS EVOKES ANTIDEPRESSANT-LIKE RESPONSES

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Depression brings about a heavy socio-economic burden worldwide due to its high prevalence and the low efficacy of antidepressant drugs. Recently, two-pore domain K+ (K2P) TASK3 channel was described as a feasible target with antidepressant potential. TASK3 knockout mice displayed antidepressant-like behaviors. However, the mechanisms or brain regions involved in these responses are not clear. Here, we developed a siRNA against TASK3 conjugated with sertraline-S (serotonin-5-HT transporter inhibitor) or reboxetine-R (norepinephrine-NE transporter inhibitor) to examine whether knockdown of TASK3 channel in the dorsal raphe nucleus (DR) or locus coeruleus (LC), respectively, is sufficient to produce antidepressant-like effects. Firstly, we infused locally naked TASK3-siRNA (10 irstly, we infused locally naked TASK3-siRNA (10) or reboxetine-R (norepinephrine-NE transporter inhi±4% and 84±4% of control group, respectively). Intranasal administration (i.n) of Alexa488-labeled S-conjugated or R-conjugated-siRNA resulted in a selective enrichment of these molecules in 5-HT TPH_2+ (DR) or NE TH+ (LC) neurons, but not in other brain regions. Moreover, short-term S-TASK3-siRNA treatment (7-day, i.n) reduced TASK3 mRNA density in TPH_2+ neurons (intracelular density: 63±7% of control group), but not TREK1 and TASK1 channels nor 5-HT_{1A} receptor and SERT. We found that DR TASK3 knockdown mice evoked antidepressant-like responses including: a) decreased immobility time in the tail suspension test, b) reduced 8-OH-DPAT-induced 5-HT1A-autoreceptor function and, c) increased fluoxetine effect on extracellular 5-HT concentration in the medial prefrontal cortex. Our results suggest that TASK3 channel in monoaminergic neurons could be a potential target for treatments of depression disorders.

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OPTIMIZATION OF AN IN VITRO BLOOD BRAIN BARRIER MODEL TO STUDY PROTECTIVE THERAPIES AFTER ISCHAEMIC STROKE AND rt-PA TREATMENT

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Introduction: Thrombolysis with recombinant tissue plasminogen activator (rt-PA) is the only approved treatment for acute ischaemic stroke. However, approximately 8% of the treated patients may suffer a symptomatic intracerebral hemorrhage due to the disruption of the blood brain barrier (BBB) and the subsequent permeability increase allowing for the pass of erythrocytes through this structure [1-3]. Our aim was to setup an *in vitro* BBB model to be used to evaluate protective BBB therapies after experimental ischaemia and rt-PA administration.

Methodology: Changes in cytotoxicity (LDH), permeability (FITC-BSA and TEER) and viability (MTT) to oxygen and glucose deprivation (OGD) during 3h and reperfusion with rt-PA during 24-48h were evaluated in the bEnd.3 endothelial cell line seeded in transwells of different manufacturers (Falcon, Corning and Millipore). In addition, a time course from 24 to 72 hours after OGD, with or without rt-PA administration, was performed using the selected transwells and analyzing FITC-BSA and MTT parameters.

Results: The optimal time to perform the OGD was 4 days after seeding of 56.700 cells/cm^2 and Falcon transwells were the best choice for our experimental conditions. OGD decreased significantly the viability of bEnd.3 cells from 24 to 72 hours and increased significantly their permeability from 24 to 48 hours. rt-PA administration only modified the permeability, which was significantly increased from 24 to 72 hours, without modifying viability.

Conclusions: After testing different experimental conditions, we have developed an *in vitro* model that could be suitable for the study of BBB protective therapies after ischaemic conditions and rt-PA treatment.

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P-45

PATHOPHYSIOLOGICAL APPROACH OF MYOFASCIAL PAIN SYNDROME IN MICE

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High anomalously spontaneous release of acetylcholine (MEEPs) seems to be responsible of the occurrence of contractions localized to a few microns of synaptic contact. These contractures are called active sites and the existence of a sufficient number of these sites is a myofascial trigger point identifiable through palpation in humans.
AIM: evaluate on mice that high concentrations of acetylcholine in synaptic cleft is responsible of active sites. Actually, we only have obtained preliminary results.
METHODOLOGY: Subcutaneous injections of anticholinesterase neostigmine were performed on adult male Swiss mice. We use the *Levator auris longus* muscle (LAL) and diaphragm. To evaluate the active sites AChRs were stained with α-bungarotoxin rodaminated in LAL muscle. To evaluate MEEPs in the LAL muscle and diaphragm we realized electrophysiological intracellular recording techniques *ex vivo*.
MAIN RESULTS: When the neostigmine is applied subcutaneously (*in vivo, in toto*): a) using α-bungarotoxin rodaminated, we have observed that 30% of synaptic contacts are contracted; b) the highest frequency of mEPPs was found in the LAL muscle (~300%, $P < 0.05$).
CONCLUSION. Whole of results obtained indicate that effectively an increases of ACh in synaptic cleft causes active sites and increases of spontaneous neurotransmission. Moreover, the subcutaneous injections of anticholinesterase seems a good manner of induce these active sites.

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SIMULTANEOUS EXPOSURE TO STRESS AND AMPHETAMINE REDUCES STRESS-INDUCED C-FOS EXPRESSION IN STRESS-RELATED AREAS

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There are scarce studies dealing with how stress and addictive drugs interact when subjects are simultaneously exposed to the two stimuli. Acute exposure to an emotional stressor and administration of d-amphetamine activates common brain areas as revealed by the expression of immediate early genes (c-fos). Some of these areas, such as the medial prefrontal cortex (mPFC) and the caudate-putamen (CPu) have important dopaminergic innervation, or play critical roles in the response of the organisms to stress (mPFC and the paraventricular nucleus of the hypothalamus, PVN). We hypothesized that simultaneous exposure to an emotional stressor and to a psychostimulant would result in a reduced stress-induced activation of some of these areas. We then studied in adult male rats brain activation caused by separate or simultaneous exposure to forced swim (FS) and to d-amphetamine (4 mg/kg), using non-radioactive *in* situ hybridization (ISH). Our results show different patterns of interaction: (i) a partially additive effect on c-fos expression in cingulate 1 region of mPFC and most subregions of the CPu, suggesting that the population of neurons activated was partially different; (ii) a lack of additive effect in infralimbic mPFC, suggesting activation of the same neurons; and (iii) a negative synergy (prelimbic mPFC and PVN). The negative synergy affected more the intensity of activation of particular neurons rather than the number of neurons and also affected corticotropin releasing factor (CRF) positive neurons in the PVN. Although the precise mechanisms involved in the negative synergy remains to be studied, the present results indicate that simultaneous exposure to FS and d-amphetamine reduces the activation of some brain areas important for the control of the response to stress and may then modify the functional consequences of stress.

P-47

ROLE OF COLLAGEN VI IN ADIPOSE TISSUE DISTRIBUTION AND FUNCTION: IMPLICATION FOR MUSCULAR DYSTROPHIES

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Ullrich Congenital Muscular Dystrophy (UCMD) and its mild form, Bethlem Myopathy (BM) are caused by collagen VI deficiency. Collagen VI is one of the most abundant proteins in the extracellular matrix of several tissues, including adipose tissue where it could contribute to the regulation of glucose and lipid metabolism. Previous microarrays studies from our laboratory have shown an increased expression of leptin and adiponectin genes in skeletal muscle from UCMD patients relative to controls. However, the possibility that genetic defects in collagen VI, in the context of muscular dystrophy, result in metabolic compromise and fat distribution changes has not been investigated.

Here, we sought to investigate whether deficiency of collagen VI leads to lipid metabolism alterations, by measuring adipokines levels in serum by enzyme-linked immunosorbent assay (ELISA), and if these alterations correlate with changes in fat mass relative to healthy population and other myopathies. Body mass composition was measured with Dual-energy X-ray absorptiometry (DXA) and various parameters were calculated such as body mass index, fat mass index and lean mass index.

UCMD and BM patients showed significantly increased levels of adiponectin and leptin in serum. Body mass composition analysis revealed a significant decrease in lean mass with a characteristic distribution and a mild increase of fat mass. Leptin and adiponectin levels showed a direct and an indirect correlation respectively with fat mass index.

These data suggest that defects in collagen VI lead to lipid metabolism impairments reflected in adipokine levels alterations and changes in fat and muscle mass distribution.

P-48

COMPARISON OF THE EFFECTS OF A SINGLE PRIOR EXPOSURE TO IMMOBILIZATION OR A COMBINATION OF VARIOUS STIMULI: THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS ACTIVITY AND CONTEXTUAL FEAR CONDITIONING

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Posttraumatic stress disorder (PTSD) is a severe anxiety disorder that develops after exposure to traumatic situations. Single-prolonged-stress (SPS) is an animal model of PTSD which consists of exposure to restraint for 2 hours, followed by 20 minutes of forced swim (FS) and, 15 minutes later, exposure to general anesthesia until loss of consciousness. This model appears to reproduce some hormonal and behavioral PTSD-like characteristics, including long-term enhanced fear conditioning. In our lab, we are studying the effects of a single exposure to immobilization on boards (IMO) as another putative PTSD model. After such exposure animals develop, over the next week, emotional and endocrine sensitization to additional stressors, but effects on fear conditioning are not consistent. It is unclear whether differences between IMO and SPS are due to the use of different rat strain or to the protocols themselves. We then decided to compare in adult male rats the effects of IMO to those of a modified SPS protocol that includes IMO instead of restraint, in order to demonstrate whether or not the SPS procedure resulted in stronger endocrine and behavioral consequences than IMO alone. Our results demonstrate that both a single exposure to IMO and the SPS paradigm (that includes IMO) induces in the short-term (24 hours) sensitization of the hypothalamic-pituitary-adrenal axis and hypo-activity in to novel environments. In the long-term (7 days), neither IMO nor SPS procedure were able to enhance shock-induced contextual fear conditioning or alter fear extinction. Interestingly, long-term reduction of the HPA response to the homotypic stressor (IMO) was similar in IMO and SPS groups, suggesting that exposure to additional stressors did not interfere with the learning-like process involved in the reduction of the HPA response to the homotypic stressor.

P-49

FINE TUNE CONTROL OF DOPAMINE NEUROTRANSMISSION BY ALPHA-SYNUCLEIN: DOWN- AND OVER-EXPRESSION MODELS

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Alpha-synuclein protein (α-syn) accumulates in the brain of patients with Parkinson´s disease (PD) and leaves a degeneration of midbrain dopamine (DA) neurons. However, the normal function of α-syn on DA neurotransmission in vivo remains poorly understood. Here, we used two mouse models with a) reduced α-syn expression in the substantia nigra compacta (SNc) and ventral tegmental area (VTA) induced by antisense oligonucleotide molecule (ASO) and, b) modest α-syn over-expression in tyrosine hydroxylase (TH)-positive neurons in the absence of overt toxicity. ASO sequence against α-syn was conjugated to a cell-specific ligand, indatraline (monoamine transporter inhibitor), to promote its selective delivery into monoamine neurons after intranasal administration. Indatraline-α-syn-ASO conjugate (1233ASO) entered into midbrain DA cells followed by trafficking to deep endomembrane vesicles associated with Rab7 resulting in an efficient α-syn knockdown. Indeed, 4-day 1233ASO treatment (30μg/day) decreased α-syn mRNA and protein levels in SNc/VTA (84.1±1.7% and 57.7±7.8% of PBS-treated animals, respectively). Alpha-synuclein suppression displayed an enhancement striatal DA tone using intracerebral microdialysis. Local veratridine (50 μM) perfusion increased extracellular DA levels more efficient in 1233ASO-treated than PBS-treated mice. Similarly, nomifensine (1-10-50 μM) or amphetamine (1-10-100 μM) showed a marked dose-effect which phenotypic differences. Tetrabenazine (VMAT2 inhibitor, 100 μM) reduced striatal DA levels in 1233ASO-treated mice. This effect was lower than in control mice. Conversely, we found that over-expressed α-syn inhibits striatal DA release. Together, this evidence indicates a physiological role for a-syn as a "fine tune" modulator of nigroestriatal DA release and the effects depend on the a-syn expression levels.

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P-50

ROLE OF TRKB NEUROTROPHIN RECEPTOR AS A HIGH AFFINITY PROTEIN RECEPTOR FOR TETANUS TOXIN

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Tetanus toxin (TeNT) acts on peripheral and CNS blocking neurotransmission and causing spastic paralysis. Its extreme neurospecificity is determined by the interaction of the carboxyl-terminal domain (H_C-TeNT) with neuronal membranes via a dual-receptor mechanism, binding first to polysialogangliosides and then to a second specific protein receptor that promotes its uptake. Although this protein receptor is not known yet, it has been reported that TeNT shares the same endocytic route that neurotrophins and their receptors. Moreover, the ability of H_C-TeNT to activate several signaling pathways dependent on Trk (Tropomyosin receptor kinase) receptors has been described. Therefore, we propose the TrkB receptor as a potential target for TeNT.

In this work we have analyzed the interaction between H_C-TeNT and TrkB receptor using a docking study to determine the regions implicated in the union. We have found that H_C-TeNT is capable to bind *in vitro* a TrkB-derived peptide of the predicted interacting region. Treatment with H_C-TeNT in cultured neurons causes phosphorylation of the TrkB receptor, suggesting an interaction between the two molecules, whereas the mutation of three residues in H_C-TeNT sequence attenuates this effect. We have analyzed also the specific binding and subsequent internalization of both the H_C-TeNT and the mutated fragment in cultured neurons by confocal microscopy and flow cytometry, finding in all cases a loss of affinity of the mutated domain.

The determination of the TeNT receptor would be a great advance in drug delivering research from the peripheral to the CNS.

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PHENCYCLIDINE ACTIVATES THALAMOCORTICAL NETWORKS BY INHIBITING GABAERGIC NEURONS IN THE RETICULAR NUCLEUS OF THE THALAMUS

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Background: The neurobiological basis of action of noncompetitive N-methyl-D-aspartate acid receptor (NMDA-R) antagonists is poorly understood. Electrophysiological studies indicate that phencyclidine (PCP) markedly disrupts neuronal activity with an overall excitatory effect and reduces the power of low-frequency oscillations (LFO; ~ 4 Hz) in thalamocortical networks[1,2]. Because the reticular nucleus of the thalamus (RtN) provides tonic feed-forward inhibition to the rest of the thalamic nuclei, we examined the effect of PCP on RtN activity, under the working hypothesis that NMDA-R blockade in RtN would disinhibit thalamocortical networks.
Methods: Drug effects (PCP followed by clozapine) on the activity of RtN (single unit and local field potential recordings) and prefrontal cortex (PFC; electrocorticogram) in anesthetized rats were assessed.
Results: PCP (.25–.5 mg/kg, intravenous) reduced the discharge rate of 19 of 21 RtN neurons to 37% of baseline ($p < .001$) and the power of LFO in RtN and PFC to ~ 20% of baseline ($p < .001$). PCP also reduced the coherence between PFC and RtN in the LFO range. A low clozapine dose (1 mg/kg intravenous) significantly countered the effect of PCP on LFO in PFC but not in RtN and further reduced the discharge rate of RtN neurons. However, clozapine administration partly antagonized the fall in coherence and phase-locking values produced by PCP.
Conclusions: PCP activates thalamocortical circuits in a bottom-up manner by reducing the activity of RtN neurons, which tonically inhibit thalamic relay neurons. However, clozapine reversal of PCP effects is not driven by restoring RtN activity and may involve a cortical action.

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INCREASING GLUTAMATERGIC TRANSMISSION IN THE RAT INFRALIMBIC CORTEX EVOKES ANTIDEPRESSANT-LIKE EFFECTS

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Recent basic and clinical studies suggest a main role of the glutamate system in the pathophysiology of depression. However, the precise mechanism by which glutamate modulates mood and emotions remains unknown. Neuroimaging studies have reported functional and metabolic alterations in the medial prefrontal cortex (mPFC) of depressed patients -particularly in its ventral regions- and these abnormalities are hypothesized to result from glial dysfunction, as shown in rodent and human studies. The current studies were conducted to determine the behavioral and neurochemical effects of glutamate modulation in the different subregions of the rat mPFC. We performed local infusions of veratridine (0.5 µl, 100 µM), a depolarizing agent, in the infralimbic cortex (IL) and the prelimbic cortex (PL) and assessed behavior in the forced swimming test (FST). We found that veratridine infusion into IL, but not in PL, produced rapid antidepressant effects. We observed similar antidepressant effects after the infusion of dihydrokainic acid (DHK) (0.5 µl, 10 mM) in IL. This agent is a GLT1 selective inhibitor that prevents astrocytic glutamate uptake. In addition, preliminary data obtained in the novelty suppressed feeding test (NSFT) also suggests antidepressant effects of DHK application into IL. This effect appears to be mediated by an increase of glutamatergic neurotransmission in IL, as assessed by in vivo microdialysis studies. Interestingly, we also found increased serotonin levels, which may be accounted for by reciprocal connectivity between the IL and the raphe nuclei. Overall, these findings suggest antidepressant-like effects after enhancing glutamatergic neurotransmission in IL.

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PHENCYCLIDINE-INDUCED DISRUPTION OF OSCILLATORY ACTIVITY IN PREFRONTAL CORTEX: REVERSAL BY ANTIPSYCHOTIC DRUGS

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The non-competitive NMDA receptor (NMDA-R) antagonist phencyclidine (PCP) is used as pharmacological model of schizophrenia due to its ability to mimic schizophrenia symptoms. PCP markedly disrupts prefrontal cortex (PFC) activity, increasing pyramidal neuron discharge and attenuating low frequency cortical oscillations (LFCO, ~1 Hz). These actions may underlie PCP psychotomimetic activity and are reversed by haloperidol and clozapine.

The aim of the present study is twofold: 1) validate the PCP-induced disruption in LFCO as a model to screen antipsychotic activity, and 2) to elucidate the pharmacological mechanisms involved in antipsychotic reversal. Classical (chlorpromazine, haloperidol, perphenazine) and atypical (clozapine, olanzapine, quetiapine, risperidone, ziprasidone) antipsychotic drugs reversed PCP-evoked loss of LFCO in rat medial PFC. Raclopride and SCH-23390 partially reversed PCP effects, suggesting that classical antipsychotic action may involve the blockade of D1-R and D2-R. 8-OH-DPAT and BAYx3702 (but not M100907) fully reversed PCP effects, suggesting that atypical drug reversal may additionally involve 5-HT1A-R activation. Pyrilamine, prazosin and citalopram were ineffective in this model.

PCP may reduce LFP by breaking the physiological balance between excitatory and inhibitory transmission in PFC, since agents enhancing GABAA-R-mediated neurotransmission, such as muscimol, diazepam and valproate were able to reverse the PCP disruption. Likewise, LY379268 (mGLuR2/3 agonist), which suppresses thalamocortical excitatory inputs onto mPFC, reversed PCP effect.

Overall, these results support the validity of the present model in antipsychotic drug screening and target identification. Moreover, the results give further support to the concept that atypical antipsychotics can exert its superior therapeutic action via 5-HT1A receptor activation, reestablishing the excitatory/inhibitory balance in PFC.

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P-54

HISTAMINE H_3 RECEPTORS NEGATIVELY MODULATE GLUTAMATE-INDUCED NEURONAL EXCITATION BY FORMING HETEROMERS WITH GLUTAMATE $MGLU_5$ RECEPTORS

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Group 1 metabotropic glutamate receptors ($mGlu_1R$ and $mGlu_5R$) are required for both persistent forms of memory and persistent synaptic plasticity, suggesting that can be target for memory and plasticity-related disorders. We hypothesized that one way to target mGluR might be via receptor heteromers. Heteromers are complexes between different receptors with biochemically distinct properties from the single receptors. Thus, we investigated potential $mGlu_5R$ partners in the hippocampus. Here by co-immunoprecipitation, Bioluminescent Resonant Energy Transfer and Proximity Ligation Assays, we report a novel receptor heteromer between $mGlu_5R$ and the histamine H_3 receptors (H_3R) in transfected cells and in rat hippocampus. A sharp inhibition of signaling by the agonist of either receptor in the presence of the agonist or the antagonist of the other receptor was seen. These results indicate a negative cross-talk in signaling when heteromers are activated with both agonists and also a cross-antagonism between receptors that might be attributed to an allosteric interaction between receptors in the H_3R-$mGlu_5R$ heteromers. Heteromer formation leads the H_3R-mediated modulation of $mGlu_5R$ signaling. The cross-antagonism and the negative cross-talk at the level of signaling in hippocampal slices were also seen at the level of extracellular field potential recordings and on pyramidal neuron Ca^{+2} mobilization and excitation. Thus, targeting H_3R-$mGlu_5R$ heteromers by H_3R ligands might be an efficient and potent way to modulate $mGlu_5R$-mediated neuronal signaling and excitability as well as neuronal plasticity. The results point out that H_3R-$mGlu_5R$ heteromers are new targets to treat neurocognitive diseases where reduced $mGlu_5R$ signaling is desired.

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REWIRING OF THE XENOPUS OLFACTORY SYSTEM THROUGH A MISTARGETED NEURONAL CONNECTION

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Neuronal circuits have little capacity to repair, as it is evident in brain or spinal cord injuries. Typically, after damage, many neurons die, synaptic connections are lost and a dramatic increase of neuronal sprouting from surviving neurons appears, in order to re-establish pre-existing connectivity. However, such newly formed processes rarely form synapses correctly inserted in a pre-existing neuronal circuit. In this work we aimed to study which factors promote *wrong* synaptic connectivity during rewiring of neuronal circuits. We used the transgenic Tubb2-GFP *X. leavis*, where the Neuronal Beta Tubulin promoter drives the expression of EGFP exclusively in the nervous system. We sectioned the olfactory nerve of Tubb2-GFP larvae at stages 45-47 and observed that about 85% of tadpoles showed a complete reconnection of the cut nerve to the bulb after 4-6 days from the lesion. Interestingly, the remaining 15% generated an aberrant ipsilateral connection between the olfactory placode and the hindbrain, which is an area specialized in gathering mechanosensitive stimuli. Time course analysis of the new nerve demonstrated that the olfactory placode-hindbrain connection was stable for more than 10 days and elimination was never observed. DiI photoconversion coupled with electron microscopy confirmed that synapses were established at the hindbrain level and revealed a morphology completely different from glomeruli. Based on these evidences, we are now investigating the molecular mechanisms generating the placode-hindbrain connection and a possible olfactory to mechanosensory change in the information conveyed by such mistargeted olfactory nerve.

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DESIGN, SYNTHESIS AND ACTIVITY OF PHOTOSWITCHABLE INHIBITORS OF DYNAMIN TO CONTROL CLATHRIN-MEDIATED ENDOCYTOSIS

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Clathrin-mediated endocytosis (CME) is an essential process operating in all cell types that regulates many physiological processes, such as synaptic transmission, internalization of pathogens, nutrients, growth factors and transmembrane receptors involved in the transduction of extracellular signals to intracellular targets.

One of the key regulatory proteins involved in CME is dynamin, a large GTPase multidomain protein involved in membrane scission during the endocytic vesicle formation.

The GTPase activity of dynamin is an attractive target to inhibit endocytosis, and a few active compounds have been recently developed. Macia *et al* [1] described a cell-permeable small molecule, 'dynasore' which inhibits dynamin endocytic function in a matter of minutes. Dynasore functions as a noncompetitive inhibitor of the GTPase activity of dynamins 1 and 2 *in vitro*, and the effects observed are reversible. The use of small chemical inhibitors, compared to other traditionally methods of dynamin inhibition, (overexpression of GTPase mutants or small interfering RNA) offers many advantages to the study of endocytosis, since the effects of these compounds are fast acting and reversible.

It would be very useful to have selective inhibitors of dynamin whose action could be remotely controlled. Optical manipulation is an appealing approach because light can be patterned with high temporal (microseconds) and spatial (microns) resolution [2].

We have designed and synthesized a series of novel photoswitchable dynasore derivatives (DynAZOs) that inhibit endocytosis in a light-dependent manner, as observed by transferrin uptake assays using cytometry and fluorescence microscopy. The compounds are cell-permeable, photo isomerize at wavelengths between 370 and 410nm, and their half-life for thermal relaxation is below 1s.

The use of photoswitchable inhibitors of endocytosis constitute a new and powerful tool to study the spatiotemporal patterns of the endocytic pathway and other signaling pathways involved in complex cellular functions where CME exerts a crucial role, such as differentiation, cell motility and invasiveness, attenuation of transmembrane receptor signaling and synaptic transmission.

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P-57

BLOCKADE OF TYROSINE KINASE RECEPTOR B PREVENTS MUSCLE CONTRACTION-INDUCED PRESYNAPTIC nPKCε, cPKCβI AND cPKCα INCREASES

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We have investigated the relation between skeletal muscle contraction, tyrosine kinase receptor B (TrkB) signaling and presynaptic expression of protein kinase C (PKC) isoforms at the adult rat neuromuscular junction (NMJ). Brain-derived neurotrophic factor (BDNF) has been identified as a contraction-induced protein produced by skeletal muscle that may contribute to the multiple health benefits associated with exercise. TrkB signaling is a key regulator of neuromuscular function. PKC family regulates a variety of neural functions, including neurotransmitter release at the NMJ. Previous studies have shown that (1) PKC family is involved in neurotransmitter release when continuous electrical stimulation imposes a moderate activity on the NMJ and that (2) muscle contraction has an important impact on levels of PKC isoforms at the NMJ. The present study is designed to test the hypothesis that the changes of expression in presynaptic PKC isoforms resulting after synaptic stimulation inducing muscle contraction at the NMJ in vivo are induced by a signaling pathway through TrkB. We use immunohistochemistry and confocal microscopy to demonstrate that nPKCε, cPKCβI and cPKCα isoforms are located in the presynaptic terminal of the adult rat NMJ. By Western blot analysis we show that blockade of TrkB prevents muscle contraction-induced nPKCε cPKCβI and cPKCα increases. These findings together with our previous result indicating that exogenous BDNF increases evoked ACh release and that trkB receptor is normally coupled to ACh release, provide mechanistic insight into how synaptic activity induced-muscle contraction could regulate the presynaptic action of the PKC isoforms linked to the neurotransmission release and suggest that muscle contraction is an important regulatory step in TrkB signaling at the NMJ.

P-58

PRESENILIN-1 REGULATES AXONAL GROWTH THROUGH RHOA IN HIPPOCAMPAL NEURONS

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Autosomal dominant mutations in the presenilin (*PS*: PS1 and PS2) account for the majority of familial Alzheimer's disease (AD) cases. PS are the catalytic subunits of γ-secretase, an enzymatic complex that cleaves transmembrane domains of type I transmembrane proteins. Recent evidence indicates that familial AD-linked mutations in PS cause reduced γ-secretase cleavage of several transmembrane proteins, suggesting a loss-of-function mechanism. Indeed, genetic inactivation of both PS in the adult brain causes synaptic plasticity and memory impairments and neurodegeneration. In this study, we analysed the biological role of PS on regulating molecular mechanisms mediating axon growth in developing hippocampal neurons. We performed immunofluorescence imaging analyses using neurofilament and dendritic/growth cone (β-actin) markers to quantify axonal growth in primary hippocampal neurons derived from control (WT) and PS1-/- knockout mouse embryos. Our results show a 50% decrease in axon length in hippocampal neurons from PS1-/- embryos. A similar result is achieved by the γ-secretase inhibitor DAPT, suggesting that the effect of PS on axonal morphology is mediated by PS/γ-secretase regulated processing of the EphA3 receptor. Due to the essential function of RhoGTPases in actin cytoskeleton reorganization during axon development, we examined whether this family of GTPases were involved on the PS-dependent regulation of axon growth. A dominant negative RhoA mutant and an inhibitor of ROCK, the effector of RhoA during actin cytoskeleton reorganization, reversed the axonal collapse in PS1-/- hippocampal neurons. In summary, our results suggest that PS mediates axon elongation in hippocampal neurons by inhibiting RhoA, whereas PS inactivation induces axon collapse.

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FUNCTIONAL VARIANTS OF THE CX3CR1 GENE ARE MODIFYING FACTORS FOR SURVIVAL AND PROGRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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Aim: To investigate the association of functional variants of the human *CX3CR1* gene (Fractalkine receptor) with the risk of Amyotrophic Lateral Sclerosis (ALS), the survival and the progression rate of the disease symptoms in a Spanish ALS cohort.

Design: Retrospective, open, naturalistic study with a cohorts of 187 ALS patients (142 sporadic [sALS] and 45 familial) and another cohort of 378 controls. *CX3CR1* V249I (rs3732379) and T280M (rs3732378) genotypes and their haplotypes were investigated as predictors of survival, progression (as measured by ALSFRS-R and FVC decline) and risk of suffering ALS.

Results: sALS patients with *CX3CR1* $249^{I/I}$ or $249^{V/I}$ genotypes presented a shorter survival time than patients with $249^{V/V}$ genotype (diff=-25.49 months; 95%CI [-42.79,-8.18]; p=0.004). The survival time was shorter in sALS patients with spinal topography and 249^{I} alleles (diff= -29.78 months; p=0.003). The same effects were also observed in the spinal sALS patients with 249^{I}-280^{M} haplotype (diff= -27.02 months; [-49.57, -4.48]; p=0.019). In the sALS group, the *CX3CR1* 249^{I} variant was associated with a faster symptoms' progression (OR=2.58; [1.32, 5.07]; p=0.006). There was no evidence for association of these two variants with ALS disease risk, age on onset or topography onset.

Conclusion: The association evidenced herein is clinically relevant and indicates that functional variants of *CX3CR1* are disease-modifying factors in sALS, affecting the progression rate of the disease's symptoms and the survival time. The 249I allele is the most potent ALS survival genetic factor reported to date. These results reinforce the role of the immune system in ALS pathogenesis.

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P-60

MDMA ACCELERATES BUT DOES NOT POTENTIATES THE PATHOGENESIS OF ALZHEIMER DISEASE.

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Background: MDMA is an amphetamine-related drug that may elicit significant neurobehavioral adverse effects, related with their mechanism in the nervous central system. The aim of the present work is to explore if MDMA can potentiate the onset of neurodegenerative traits of a transgenic strain that mimics the features of Alzheimer's disease (AD).

Methods: Male mice C57BL/6, wild type (WT) or double transgenic APPswe/PS1dE9 (APP), 1-month-old were treated using the following dose schedule: 3 s.c. doses/day (3 h), once/week for 8 weeks. MDMA: 5 mg/kg (2 weeks)-7.5 mg/kg (3 weeks)-10 mg/kg (3 weeks). When animals were 3 or 6 month old underwent the Object Recognition Test (ORT). Glial fibrillary acidic protein (GFAP), Iba1, a marker of microgliosis, and Phospho-amyloid precursor protein (p-APP) immunofluorescences were performed. Amyloid-β deposition was detected by Thioflavin S staining. The levels of soluble and insoluble Aβ40/42 in the brain of APPswe/PS1dE9 mice were quantified by Elisa Kit.

Results: Both WT and APP MDMA-treated animals at 3 months old lost memory 24h after the familiar session in the OF that did not recover it at 6 months old. MDMA facilitated the plaque deposition in transgenic mice at 3 months old that ran in parallel with an increase of insoluble Aβ40/42 levels in cortex. Accordingly, there was an increase of both p-APP expression and GFAP that were surrounding the plaques. There were no changes in Iba-1 expression. All these features got equalized at 6 months old between MDMA treated animals and their controls.

Conclusions: In transgenic APPswe/PS1dE9 mice, MDMA accelerates the onset of pathognomonic signs of AD, but doesn't potentiates it throughout time.

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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 clinical feature of Huntington's disease (HD) which has gradually gained importance over the classical motor symptoms of the pathology. 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 point out therapeutic strategies aimed to inhibit 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 cortico-striatal learning deficits and improved performance in spatial and memory learning tasks, which suggests that alterations in both cortico-striatal and hippocampal functions in HD could involve aberrant Cdk5 activity. Moreover, our data suggests that improved cognition in Hdh$^{Q7/Q111}$; Cdk5$^{+/-}$ mice could be through modulation of the Cdk5/Src/pTyr1472-GluN2B pathway. Altogether, these findings demonstrate that modulation of Cdk5 activity or signalling in HD may contribute to restore synaptic plasticity and learning defects in this devastating disorder.

P-62

CRTC1 NUCLEAR TRANSLOCATION IS CRITICAL FOR HIPPOCAMPAL-DEPENDENT ASSOCIATIVE MEMORY

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Associative learning deficits are common in dementia patients but the molecular mechanisms underlying impairments of associative emotional memory in neurodegeneration remain largely unknown. In this study, we examined the effects of context-dependent learning on nuclear translocation and function of the transcriptional effector CREB regulated transcription coactivator 1 (CRTC1). By using fear conditioning, we found that contextual fear learning triggers rapid translocation of CRTC1 from the cytosol and dendrites to the nucleus of CA3 hippocampal neurons. CRTC1 nuclear translocation by context fear learning is associated with CRTC1 dephosphorylation at Ser151, a residue critical for transcriptional activation. Contextual fear learning induces a differential expression of the CREB target genes, including the *Nr4a* family members *Nr4a 1, 2 and 3*. Notably, reduced CRTC1 nuclear translocation and transcription is associated with long-term memory deficits in a mouse model of neurodegeneration lacking the presenilin genes Psen1 and Psen2, while adeno-associated viral-mediated CRTC1 overexpression rescues contextual fear memory and transcriptional deficits in these presenilin mutant mice. Taking together, our results suggest a critical role of CRTC1 nuclear translocation and transcriptional function in contextual memory encoding, and provide evidence that enhancing CRTC1 function ameliorates associative memory deficits during neurodegeneration.

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ALPHA-SYNUCLEIN/MITOCHONDRIA INTERACTION ALTERS MITOCHONDRIAL PROTEIN IMPORT

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Alpha-synuclein (α-Syn) accumulation and mitochondrial dysfunction play prominent roles in the pathology of Parkinson's disease (PD). A fraction of α-Syn interacts with mitochondria, but the consequences of α-Syn–mitochondria interactions in the normal and pathologic effects of α-Syn remain poorly defined.

In neuroblastoma BE-M17 cells, our results show that α-Syn is translocated to mitochondria after complex I inhibition. Interestingly enough, induction of complex I inhibition by the mitochondrial toxin MPP^+, in cells overexpressing α-Syn, diminishes the interaction of α-Syn with mitochondria. Moreover, complex I inhibition and α-Syn overexpression markedly increased protein import of ^{35}S-labeled proteins.

In vitro as well as in vivo, in the midbrain of MPTP-intoxicated brain of mice, the integrity of the mitochondrial protein import is affected. Indeed, our data indicate that the level of expression of Tim 23, a protein involved in the mitochondrial import system of the inner membrane was decreased by 50% as early as 1 day after the last MPTP injection and maintained until 7 days after the last injection. Our data indicate that the import system through the inner membrane of mitochondria, after inhibition of complex I, is affected and can contribute to mitochondrial dysfunction associated with dopaminergic neurodegeneration in Parkinson's disease. Although further experiments are necessary, we hypothesis that altered mitochondrial protein content accompanied by selective increases in protein import into mitochondria, which may be associated with increased aggregates inside the mitochondria and mitochondrial quality control dysfunction, might be part of the mitochondrial damage arising from complex I inhibition and α-Syn accumulation in mitochondria.

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7,8 DIHYDROXYFLAVONE (7,8-DHF) AMELIORATES COGNITIVE AND MOTOR DEFICITS IN A HUNTINGON'S DISEASE MOUSE MODEL THROUGH A DIFFERENT ACTIVATION PROFILE FROM BDNF

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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 abnormal motor coordination, cognitive decline and psychiatric disorders; the pathophysiology includes reduced levels of brain-derived neurotrophic factor (BDNF); thus BDNF activation through its high-affinity receptor TrkB has become of interest for its therapeutic implications. 7,8-Dihydroxyflavone (7,8-DHF) has been described as a TrkB receptor agonist in a variety of in vitro models and tested on in vivo models of neurodegenerative diseases. It has been described that chronic treatment with 7,8-DHF can ameliorate motor deficits in N171-82Q HD mice; here we tested its effects on the R6/1 mouse model of HD. We found chronic treatment with 7,8-DHF is able to delay the onset of motor deficits in R6/1 mice assessed by the Rotarod test; furthermore 7,8-DHF reversed the inability to perform correctly the Novel Object Recognition Test (NORT) at 15 weeks; pathological and biochemical analyses of treated mice revealed improved levels of Enkephalin in striatum, (concordant with a trend on a DARPP32 recovery) and a reduction of striatal volume loss upon treatment. Interestingly in vivo results showed $TrkB^{Y816}$ but not $TrkB^{Y515}$ phosphorylation recovery in striatum with acute and chronic treatment. 7,8-DHF treatment in primary neuronal cultures revealed the same differences in TrkB phosphorylation profile and morphologic divergent changes from controls with BDNF. 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.

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FINGOLIMOD (FTY720) ENHANCES HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY IN HUNTINGTON'S DISEASE BY PREVENTING $P75^{NTR}$/TRKB IMBALANCE

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Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by motor and cognitive impairments. Striatal atrophy is the main pathological hallmark, but degeneration in other regions of the brain, like the cerebral cortex and the hippocampus, has been reported. HD mouse models display low levels of brain-derived neurotrophic factor (BDNF) and altered expression of its receptors TrkB and $p75^{NTR}$, which have been involved in the regulation of synaptic plasticity and cognitive function. Fingolimod (FTY720), an agonist of sphingosine-1 phosphate receptors commonly used as an immunomodulator in Multiple Sclerosis patients, has recently been shown to increase BDNF levels. Here, we have investigated whether FTY720 improves synaptic plasticity and memory dysfunction in the R6/1 mouse model of HD through regulation of BDNF signaling. Chronic administration of FTY720 from pre-symptomatic stages prevented long-term memory deficits and dendritic spine loss in CA1 hippocampal neurons from R6/1 mice. FTY720 increased BDNF mRNA in the hippocampus, without altering pro-BDNF processing. However, FTY720 chronic treatment prevented $p75^{NTR}$ up-regulation and promoted TrkB phosphorylation in the hippocampus of R6/1 mice, supporting a role for FTY720 in the enhancement of synaptic plasticity. FTY720 modulated $p75^{NTR}$ expression likely by decreasing astrogliosis and tumor necrosis factor α (TNFα) levels within the hippocampus of R6/1 mice. Our findings define a new mechanism for the action of FTY720 in neurodegenerative diseases and propose this drug as a suitable candidate for treating cognitive dysfunction in HD.

P-66

EFECTES DEL PÈPTID β-AMILOIDE A LA VIA SECRECIÓ REGULADA EN NEURONES I ASTRÒCITS

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El sistema de secreció regulada és clau pel manteniment de la funció normal del sistema nerviós central, ja que controla l'alliberament de neuropèptids, hormones peptídiques i factors de creixement. S'han observat alteracions en els nivells d'aquestes substàncies bioactives en pacients de la malaltia d'Alzheimer, el que podria suggerir que la via de secreció regulada és una possible diana de la patologia. Aquí presentem els efectes del pèptid β-amiloide sobre dos marcadors de la via de secreció regulada, la Carboxipeptidasa E i la Segretogranina III. Els agregats del Aβ1-42 es van preparar i caracteritzar per WB i es va analitzar el seu efecte diferencial sobre cultius enriquits en neurones i astròcits. La incubació amb aquest pèptid neurotòxic va provocar una disminució en l'alliberament de SgIII i CPE al medi extracel·lular, de manera basal en astròcits i de forma depenent d'estímul en els cultius neuronals analitzats. Per avaluar l'especificitat d'aquest efecte es va comprovar que la viabilitat no estigués afectada i es va analitzar també l'alliberament d'un neurotransmissor clàssic -glutamat-, que es va veure augmentada amb el tractament. Aquests resultats suggereixen una alteració en la via de secreció regulada en resposta al pèptid β-amiloide, el que podria indicar que aquesta via està alterada en etapes primerenques de la malaltia d'Alzheimer.

P-67

BRAIN STUDIES IN A HUMAN FETUS WITH TYROSINE HYDROXYLASE DEFICIENCY

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Background and objectives: Tyrosine Hydroxylase (TH) Deficiency (THD) "B" phenotype produce a severe encephalopathy with sub-optimal L-dopa response. Our aim was to investigate the expression of some synaptic proteins in a THD fetal brain carrying mutations that produce a "B" phenotype in her sister.

Material: Brain tissue of a 16-week fetus miscarriage was dissected and immediately frozen at -80ºC. TH genetic study detected p.R328W/p.T399M mutations (as her sister affected by a severe "B" phenotype). TH, VMAT1, VMAT2, AADC, D1DR, D2DR, CALBINDIN, GABAVT, NMDAR1, PSD95, SMI31, MAP2, PSD95 and NSE were quantified by Western blot in the different cortical areas, mesencephalon, protuberance, cerebellum and suprarenal gland (SG). Results were compared with an age-matched control fetus.

Results: TH, VMAT1, VMAT2 were under-expressed in all sections and the suprarenal gland. AADC was differentially expressed. Both dopamine receptors (D1DR and D2DR) were under-expressed in all brain sections. Calbindin and NMADAR1 were stably expressed among the brain regions with some exceptions. A reduction of GABAVT was observed in all brain sections. PSD95 and SMI31 were mostly under-expressed, whereas MAP2 was over-expressed in all the sections. No differences were observed on the NSE expression.

Discussion: The observed changes in the expression of these proteins suggest the role of Dopamine as an important regulator of other neurotransmitter systems and brain development. We are currently studying other biomarkers to better understand neurodevelopmental abnormalities in this disease.

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PROTECTIVE CAPACITY OF C-TERMINAL FRAGMENT OF TETANUS TOXIN IN $APP_{Swe,Ind}$ TRANSGENIC MICE

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Several therapeutic strategies have been proposed to prevent Alzheimer's disease (AD). The neurotrophins are a family of growth factors present in the central nervous system (CNS), which activate survival pathways by their binding to tropomyosin-related kinase receptors. They are also involved in memory acquisition and retention, notably in hippocampal areas. Alterations in levels of neurotrophins and their respective receptors have been found in AD. Therefore, neurotrophins might have therapeutic relevance in AD, but there are several disadvantages that complicate their administration. The C-terminal fragment of tetanus toxin (Hc-TeTx) has the ability to enter the CNS by retroaxonal transport where it activates the same signaling cascades as neurotrophins. In addition, Hc-TeTx is able to elude many of the problems associated with neurotrophin administation, and might therefore be used as a therapeutic agent in AD treatment. The objective of the present study was to evaluate the neuroprotective capacity of Hc-TeTx in early AD pathology using the trangenic $APP_{Swe,Ind}$ mouse model. For this purpose, confocal imaging of the hippocampus was used to compare pathological markers in $APP_{Swe,Ind}$ mutant mice and wild type (WT) mice, with and without previous administration of Hc-TeTx. In addition, Western blot analysis was performed in order to try to elucidate the molecular mechanisms by which Hc-TeTx might exert its neuroprotective effect. The results show a significant reduction of β-amyloid (Aβ) levels in the hippocampal cornu ammonis 1 (CA1) region in $APP_{Swe,Ind}$ mice treated with Hc-TeTx. Hc-TeTx might therefore be used in early stages of AD to halt disease progression.

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OVEREXPRESSION OF TRANSCRIPTION FACTOR EB PREVENTS DOPAMINERGIC CELL DEATH IN THE MPTP MOUSE MODEL OF PARKINSON DISEASE

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Parkinson disease (PD) is a common neurodegenerative disorder mainly characterized by the loss of dopaminergic neurons from the substantia nigra pars compacta (SNpc). Although the cause of PD is unknown, mounting evidence indicates that alterations in autophagy-lysosome pathway may be involved.

We have previously shown that in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, dopaminergic cell death is preceded by a marked decrease in the amount of lysosomes and also by the subsequent accumulation of undegraded autophagosomes (AP) within dopaminergic neurons. Induction of lysosomal biogenesis by overexpression of transcription factor EB (TFEB) by means of recombinant adenoassociated viral vector (rAAV) restored lysosomal levels, increased AP clearance and attenuated MPP^+, MPTP's active metabolite, cell death *in vitro*.

Hence, we assessed whether overexpressing TFEB in the ventral midbrain using a rAAV can be neuroprotective in the MPTP mouse model of PD. Our results show that the rAAV used to overexpress TFEB is able to efficiently transduce dopaminergic nigral cells *in vivo* and preventing the PD-like neurodegenerative changes induced by MPTP neurotoxin, such as cell death at the level of substantia nigra cell bodies and denervation of striatal axon terminals. Furthermore, lysosomal depletion and AP accumulation within dopaminergic neurons in MPTP-intoxicated mice were also prevented by TFEB. Therefore, therapeutic strategies aimed at rescuing or enhancing lysosomal and autophagic function may thus represent a novel neuroprotective strategy in PD.

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COMPARISON OF HIPPOCAMPAL PHENOTYPES IN C57BL/6 WILD-TYPE, APP/PS1 AND LDLr KNOCKOUT MICE

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A number of studies suggest that there is a link between Alzheimer disease (AD) and cholesterol metabolism impairments. Altered intracellular compartmentalization of cholesterol in the brain has been implicated in increased amyloid-beta (Aβ) peptide generation. In addition, significant cognitive impairments and memory loss were described in mice lacking low-density lipoprotein receptor (LDLr $^{-/-}$), a model of familial hypercholesterolemia. LDLr is partly responsible for receptor-mediated endocytosis of Apolipoprotein E, one of the isoforms of which (apoε4), is a major genetic risk factor for AD. For this reason, we compared the hippocampal phenotypes of LDLr$^{-/-}$ mice with a transgenic mouse model of familial AD (APP/PS1), focusing our research on the analysis of molecular mechanisms related to memory processes, oxidative stress and inflammation. 6 months old male mice were chosen for this study because at this age, a significant memory loss is present in both models. We have detected a slight, but significant increase in mRNA and protein levels of molecules related to oxidative stress in the brains of both APP/PS1 and LDLr$^{-/-}$ animals, when compared to wild-type controls. Interestingly, elevated expression of proinflammatory markers such as IL-1b, IL-6 and TNFα was observed in APP/PS1 brains but not in the hippocampal extracts of LDLr$^{-/-}$ animals, contrary to some reports. In summary, our data suggest that the molecular signalling pathways implicated in memory loss are distinct in APP/PS1 and LDLr$^{-/-}$ mouse models.

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THE EFFECTS OF A HIGH-FAT DIET IN AN APP/PS1 MOUSE MODEL OF ALZHEIMER DISEASE

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Alzheimer disease (AD) is a progressive neurodegenerative disease which affects adult brain. One of the challenges in AD research is to identify early markers leading to cognitive decline and neuronal loss. This is especially important, as by the time the clinical signs of dementia appear, it may already be too late to correct the course of the disease. Recent evidence from experimental and clinical studies suggests that altered brain glucose metabolism may be present well in advance of clinically measurable cognitive loss. In the present work, we have studied the effects of a high fat-rich diet in an APP/PS1 transgenic mouse model of familial AD. Male C57BL/6 wild-type and APP/PS1 animals were switched to a 45 kcal% fat diet consisting mainly of hydrogenated coconut oil at the time of weaning. Mice were sacrificed at 6 months of age – a timepoint at which APP/PS1 animals present with brain amyloid-beta plaque deposits and memory loss. We have observed significant alterations in peripheral metabolic parameters including glucose, insulin and triglycerides levels, as well as impaired glucose and insulin tolerance in high-fat-fed APP/PS1 animals. At the CNS level, a high-fat diet resulted in an increase in the insoluble Aβ42 in the brains of APP/PS1 animals, and caused significant changes in the hippocampus at the molecular level. In summary, our data demonstrate that the peripheral metabolic phenotype induced by a high-fat chow results in alterations in hippocampal signaling pathways related to energy homeostasis and may provoke accelerated AD progression in a mouse model of AD.

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