XI SIMPOSI DE NEUROBIOLOGIA

FUTURE TECHNICAL ADVANCES

12 i 13 de novembre de 2018

Institut d'Estudis Catalans, Barcelona

Programa i resums de les comunicacions

Amb el patrocini de:

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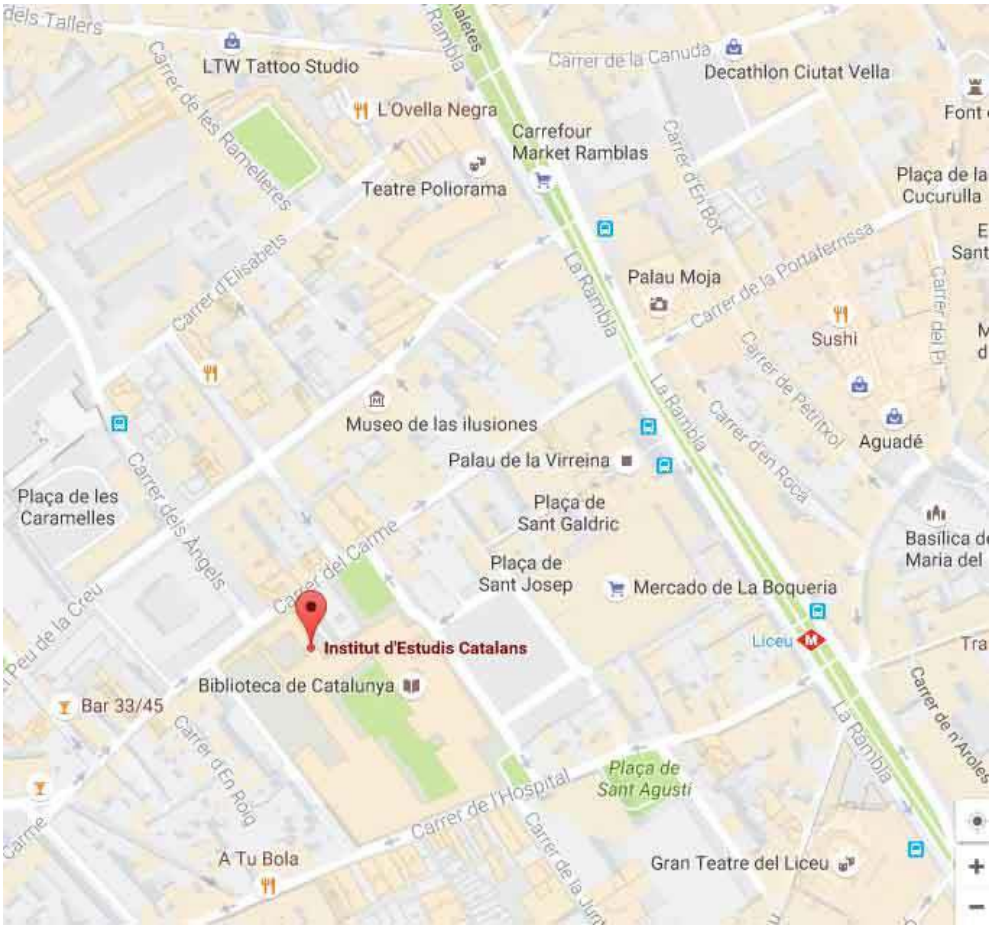

INSTITUT D'ESTUDIS CATALANS
Carrer del Carme, 47
Barcelona 08001

Dia 1: 12 Novembre de 2018		Dia 2: 13 Novembre de 2018	
8.30-8.50 h	**Registre i recollida de material**		
8.50 h	**Benvinguda i presentació del simposi** Sala: Prat de la Riba		
9.00-10.30h	**Sessió 1A** Oral **Cèl.lules glials i inflammació** Sala: Prat de la Riba **Sessió 1B** Oral **Neurodesenvolupament i cél.lules mare nerviós** Sala: Pere i Joan Coromines	9.00-10.45 h	**Sessió 3A** Oral **Circuits neuronals i plasticitat** Sala: Prat de la Riba **Sessió 3B** Oral **Receptors de neurotransmissors i neurofarmacologia II** Sala: Pere i Joan Coromines
10.30-12.00 h	Cafè i sessió de **pòsters P1-64**	10.45-12.00 h	Cafè i sessió de **pòsters P1-64**
12.00-13.00 h	**Conferència plenària 1** **Lluis Montoliu** **Centro Nacional de Biotecnologia (CNB-CSIC), Madrid** Títol: «DNA editing in biomedicine research and therapy » Sala: Prat de la Riba	12.00-13.00 h	**Conferència plenària 3** **Andrea Volterra** **Université de Lausanne, Switzerland** Títol: «Towards decoding the language of astrocytes via 1D to 3D Ca^{2+} imaging» Sala: Prat de la Riba
13.00-14.45h	**Dinar i sessió de pòsters P1-64**	13.00-14.45 h	**Dinar** i sessió de **pòsters P1-64**
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16.30-17.30 h	**Xerrades breus dels pòsters:** P6 Masgrau R P17 Companys-Alemany J P36 Bueno Fernández C P52 Rabaneda-Lombarte N P61 Edo A 8 min cadascuna Sala: Prat de la Riba	16.30-17.30 h	**4t Premi Ramon Turró** Conferència d'acceptació **Jesús Pujol** **Unitat de Recerca en Neuroimatge, Hospital del Mar, Barcelona** Títol: «Quan finalitza el desenvolupament del cervell humà?» Sala: Prat de la Riba
		17.30-17.45 h	**Premis Millors Pòsters** **Clausura**
		17.45-20.00 h	**FESTA DE CLAUSURA**

10 min presentation + 5 min questions

Day 1. November 12th, 2018

8.30-8.50 h	Registration
8.50 h	**Welcome presentation** Room: Prat de la Riba
9.00-10.30 h	**Session 1A** Oral **Glial cells and inflammation** Room: Prat de la Riba **Session 1B** Oral **Neurodevelopment and stem cells** Room: Pere i Joan Coromines
10.30-12.00 h	Coffee and **poster session P1-64**
12.00-13.00 h	**Plenary lecture 1** **Lluis Montoliu** **Centro Nacional de Biotecnologia (CNB-CSIC), Madrid** Títol: « DNA editing in biomedicine research and therapy » Room: Prat de la Riba
13.00-14.45 h	**Lunch and poster session P1-64**
14.45-16.30	**Session 2A** Oral **Neurodegenerative diseases I** Room: Prat de la Riba **Session 2B** Oral **Neurotransmitter receptors and neuropharmacology I** Room: Pere i Joan Coromines
16.30-17.30 h	**Short talks of the posters:** P6 Masgrau R P17 Companys-Alemany J P36 Bueno Fernández C P52 Rabaneda-Lombarte N P61 Edo A 8 min each one Room: Prat de la Riba

10 min presentation + 5 min questions

Day 2. November 13th, 2018

9.00-10.45 h	**Session 3A** Oral **Neuronal circuits and plasticity** Room: Prat de la Riba **Session 3B** Oral **Neurotransmitter receptors and neuropharmacology II** Room: Pere i Joan Coromines
10.45-12.00 h	Coffee and **poster session P1-64**
12.00-13.00 h	**Plenary lecture 3** **Andrea Volterra** **Université de Lausanne, Switzerland** Títol: «Towards decoding the language of astrocytes via 1D to 3D Ca^{2+} imaging» Room: Prat de la Riba
13.00-14.45 h	**Lunch and poster session P1-64**
14.45-16.30 h	**Session 4A** Oral **Neurodegenerative diseases II** Room: Prat de la Riba
16.30-17.30 h	**4th Ramon Turró Award Conference** **Jesús Pujol** **Unitat de Recerca en Neuroimatge, Hospital del Mar, Barcelona** Tittle: «Quan finalitza el desenvolupament del cervell humà?» Room: Prat de la Riba
17.30-17.45 h	**Best Posters Awards** **Closing remarks**
17.45-20.00 h	**ENDING PARTY**

DAY 1: Monday, November 12th 2018

8.30-8.50	**REGISTRATION**
8.50	**SYMPOSIUM PRESENTATION and WELCOME SPEECH** *Prat de la Riba room* Carles A. Saura
9.00-10.30	**ORAL SESSIONS** **Session 1A. GLIAL CELLS and INFLAMMATION** *Prat de la Riba room* **Chairwoman: Carme Solà (CSIC, IDIBAPS)** O.1. Regionally-selective knockdown of astroglial glutamate transporters in infralimbic cortex increases local excitatory neurotransmission and evokes a depressive phenotype in mice **Fullana MN** O.2. Interaction between GPR18 and cannabinoid CB2 G-protein-coupled receptors: relevance in neurodegenerative processes **Reyes-Resina I** O.3. P2RY12 inhibition in microglia reduces the phagocytosis of neurons after an excitotoxic injury **Petegnief V** O.4. CCR2+ Monocytes promote recovery following stroke in mice **Pedragosa J** O.5. Classification of microglial cells based on multiple parameters measurements: a live imaging study **Martinez A** O.6. Gut brain-axis in stroke: intestinal immune system is altered by surgical stress in the mouse model of cerebral ischemia/reperfusion **Diaz L** **Session 1B. NEURODEVELOPMENT AND STEM CELLS** *Pere i Joan Coromines room* **Chairman: Andrés Míguez (UB, INC, IDIBAPS)** O.7. Studying axon guidance and neuronal migration with selective interference of second messenger function in vivo **Ros O**

	O.8. Identification of striatal progenitor sub-populations during human embryonic stem cell differentiation using single-cell RNAseq **Sanders P** O.9. Modeling tyrosine hydroxylase deficiency using induced pluripotent stem cells **Tristán-Noguero A** O.10. Human iPSC-mouse chimeras to study Huntington's disease phenotypes **Miguez A** O.11. Neurodevelopmental alterations in Huntington's disease **Vila C** O.12. Deciphering NMDAR mutations pathogenicity in neurodevelopmental conditions **Santos-Gómez A**
10.30-12.00	**COFFEE BREAK and POSTERS**
12.00-13.00	**PLENARY LECTURE** *Prat de la Riba room* **« DNA editing in biomedicine research and therapy »** Lluís Montoliu (Centro Nacional de Biotecnología, CNB - CSIC, CIBERER-ICIII, Madrid)
13.00-14.45	**LUNCH**
14.45-16.30	**ORAL SESSIONS** **Session 2A. NEURODEGENERATIVE DISEASES I** *Prat de la Riba room* **Chairwoman: Eulàlia Martí (UB, IDIBAPS)** O.13. Presenilin regulates tau phosphorylation and inflammation during neurodegeneration **Soto-Faguás CM** O.14. Neuroprotective mechanisms of resveratrol in a high-fat diet-fed Alzheimer's disease mouse model **Gatius A** O.15. RNA-Seq differential expression analysis of dendritic cells and microglia in the ischemic brain tissue of mice **Gallizioli M**

	O.16 Molecular overview of an amyotrophic lateral sclerosis mice model: an insight to the BDNF/TRKB signaling pathway and its coupled PKCS and SNARE/SM targets **Borràs L** O.17 Targeting JNK1 as a method to improve metabolic-derived cognitive deficits **Busquets O** O.18 Severe cortical affectation after complex i subunit deletion in CCK-expressing cells **Urpi A** O.19 Altered functional connectivity and network dynamics in huntington's disease striatal primary cultures reveales through large-scale calcium imaging **Fernández-García S** **Session 2B. NEUROTRANSMITTER RECEPTORS and NEUROPHARMACOLOGY I** *Pere i Joan Coromines room* **Chairman: David Soto (UB, INC, IDIBAPS)** O.20. Dopamine D_2-like autoreceptor partial agonist antipsychotics decrease dopamine synthesis **Omar MY** O.21. *In vivo* photomodulation of gaba and glycine receptor channels **Gomila A** O.22. Photoswitchable dynasore analogs to control endocytosis with light **Camarero N** O.23. Effect of pridopidine, a dopamine stabilizer, on the phencyclidine-based animal model of schizophrenia **Valle-León M** O.24 Chronic adenosine A2a receptor blockade induces locomotor sensitization and potentiates striatal ltd in GPR37 deficient mice **Morató X** O.25. Regulation of AMPAR trafficking by CPT1C depalmitoylation **Gratacòs-Batlle E**
16.30-17.30	**SHORT TALKS OF THE POSTERS** P6 Masgrau R P17 Companys-Alemany J P36 Bueno Fernández C P52 Rabaneda-Lombarte N P61 Edo A 8 min each one *Prat de la Riba room*

DAY 2: Tuesday, November 13th 2018

9.00-10.45	**ORAL SESSIONS** **Session 3A. NEURONAL CIRCUITS and PLASTICITY** *Prat de la Riba room* **Chairman: Albert Giralt (UB, INC, IDIBAPS)** O.26. Carnitine palmitoyltransferase-1 deletion in AGRP neurons increases energy expenditure by enhancing brown adipose tissue activity **Serra D.** O.27. Optogenetic stimulation of corticostriatal pathway ameliorates motor behavior in Huntington's disease **Masana M.** O.28. The migatte no gokui state in mice is hampered by the hippocampus **Giralt A** O.29 The role of ZNF804A and AKT1 genes and cannabis use in psychosis risk: a gene-environment interaction study in a non-clinical sample **Soler J** O.30. Postnatal exposure to chlorpyrifos differently affects associative recognition memory depending on sex and apoe genotype **Guardia-Escote L** O.31. Genetic variability in neural excitability genes modulates cognitive performance and brain activity. a case-control study in schizophrenia **Guardiola M** O.32. The role of *neuritin gene* in modulating schizophrenia age at onset and brain activity during a working memory task **Almodóvar C** **Session 3B. NEUROTRANSMITTER RECEPTORS and NEUROPHARMACOLOGY II** *Pere i Joan Coromines room* **Chairman: Coral Sanfeliu (IIBB, CSIC, IDIBAPS)** O.33. Role of the transcription factor Nr4a2 on glutamatergic synapses in the hippocampus **Català-Solsona J** O.34. Functional impact of DYRK1A-mediated phosphorylation of the NMDA receptor **Locubiche-Serra S**

	O.35. Alterations in GABA A1 receptor in Rett syndrome: the necessity of early GABAergic modulation as a therapeutic strategy **Oyarzabal A** O.36. Traffic lights peptides: a tool to photocontrol clathrin-mediated endocytosis **Prischich D** O.37. Chronic ototoxicity of the inner ear causes a loss of synapses and functionality in mice **Greguske E** O.38. Dissecting the vestibular circuitry of motion sickness **Machuca-Marquez P**
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13.00-14.45	**LUNCH and POSTERS**
14.45-16.30	**ORAL SESSION** **Session 4A. NEURODEGENERATIVE DISEASES II** *Prat de la Riba room* **Chairman: Assumpció Bosch (INc, UAB)** O.39. Involvement of small RNAs in the pathophysiology of Huntington's disease: focus on sCAG species **Guisado-Corcoll A** O.40. Cerebellar transduction of astrocytes as gene therapy strategy for Megaloencephalic Leukoencephalopathy with subcortical Cysts (MLC) **Sanchez, A** O.41. Increased lamin B1 levels contribute to alterations in nuclear permeability in Huntington's disease **Castany-Pladevall C**

	O.42. DRP1-mitochondrial fragmentation contributes to disrupting mitochondria-ER contact sites in Huntington's disease mouse striatum **López-Molina L** O.43. Role of neuromelanin in Parkinson's disease **Carballo-Carbajal I** O.44. Analysis of epigenetic alterations in SAMP8, a model of age related cognitive decline **Griñán-Ferré, C** O.45. Efectos de APOE sobre la cognición y su interacción con factores demográficos y BDNF **Lamonja N**
16.30-17.30	**4th RAMON TURRÓ AWARD** honoring the most cited article in Neurosciences performed in Catalunya published 25 years ago (1992-93) for the publication "Pujol et al (1993) When does human brain development end? Evidence of corpus callosum growth up to adulthood. Ann Neurol 34; 71-75" *Prat de la Riba room* **ACCEPTANCE LECTURE** **« Quan finalitza el desenvolupament del cervell humà ? »** Jesús Pujol (Hospital del Mar, Barcelona)
17.30-17.45	**BEST POSTERS AWARDS** to the 3 best posters presented at the XI Symposium *Prat de la Riba room* **CLOSING SPEECH** Carlos A. Saura
17.45-20.00	**BEER and MUSIC. THE NEVER-ENDING NEUROSCIENCE PARTY**

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RESUMS
DE LES COMUNICACIONS ORALS
I PÒSTERS

PLENARY LECTURES

L.1. DNA EDITING IN BIOMEDICINE RESEARCH AND THERAPY

Montoliu Ll

Centro Nacional de Biotechnología (CNB), CSIC, CIBERER-ISCIII, Madrid, Spain

Genome editing tools have changed the current scenario at many biology and biomedicine laboratories. Using CRISPR-Cas tools, derived from an ancient adaptive immune system developed by prokaryotes, we can now address almost any type of genome modification, at will, with an efficacy that has not been observed before. These are affordable, easy to use and access and flexible tools which can be adapted to all needs and aims, from deletions to insertions, from substitutions to inactivations of genes. It is now possible to reproduce mutations found upon genetically diagnosing patients into cellular and animal models, in order to investigate the ethiology and the causes and consequences of these genetic alterations. Furthermore, one can envisage advanced gene therapy approaches based on CRISPR-Cas tools, where one can attempt to correct and fix a given specific mutation and substitute it by its corresponding wild-type sequence. The progress is spectacular and there is not a single week where no novel innovative applications are released. It is also horizontally impacting on biomedicine projects, in all fields, including Neurobiology. Neurodegenerative and neurological disorders, mostly untreatable and without cure, can have some hope now thanks the current genome editing hype. However, one must be honest and accept our current limitations in our understanding of the genome editing process. At present, we still cannot decide which allele is going to be produced and we have to invest time and resources into sequencing and segregating different allelic variants before we come across the one we are interested in. I will be presenting the current state-of-art of CRISPR-derived genome editing methods, with their pros- and cons- and will be discussing some future developments where we expect some substantial improvements to be occurring in the near future.

NOTES

L.2. IMAGING NEUROGLIAL CROSSTALK: NATURE AND FUNCTION

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Brain information processing is commonly thought to be a neuronal performance. However numerous data now point to a key role of astrocytes in brain development, activity and pathology. Indeed astrocytes are now viewed as crucial elements of the brain circuitry that control neuronal circuits formation, maturation, activity and elimination. How do astrocytes exert such control is a matter of intense research that is crucial not only for our understanding of brain development and function, but also for identifying novel therapeutic targets. Imaging the neuroglial crosstalk has been and is still one of the major approaches used to unravel how astrocytes participate to neural circuits modeling. In the last years, major technical advances have been performed. I will here present some of them, pointing to the evolution of our comprehension of the neuroglial crosstalk. I will particularly focus on the different forms it can take and its multiple functions. Heterogeneity and versatility in the astroglial regulations of neuronal functions will also be addressed.

NOTES

L.3. TOWARDS DECODING THE LANGUAGE OF ASTROCYTES VIA 1D-TO-3D Ca^{2+} IMAGING

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Astrocytes sense neuronal inputs and in turn modulate synapses and blood vessels via intracellular Ca^{2+} signaling. Despite their importance, the properties of astrocytic Ca^{2+} signals are not well understood, also because studied with conventional 2D imaging, which monitors ~ 5% of an astrocyte volume. We developed 3D Ca^{2+} imaging of entire individual astrocytes in adult hippocampal slices and *in vivo* in awake mice using GCaMP6f (Bindocci et al., Science, 2017). We routinely acquired two full z-stacks of ~60 focal planes/sec, capturing all Ca^{2+} events lasting ≥1.5 sec (FWHM), and visualized faster events in 10Hz acquisitions (8 planes/sec). Concerning distribution, 80% of an astrocyte Ca^{2+} activity was localized in the optically sub-resolved part of the cell (the so-called gliapil), with the remaining 20% in the structural core (soma, stem processes, end-feet). Core activity was mostly in processes and end-feet, whereas somatic activity was infrequent and not representative of the astrocyte Ca^{2+} dynamics. 10Hz acquisitions showed that activity in processes is mainly fast (FWHM:~0.7 sec) and local (~40 um3, ~12% of the process volume), resembling in size the events produced by minimal axonal stimulations (~60 um3). Likewise, Ca2+ activity in end-feet is mostly confined to the end-foot domain itself and contains fast (FWHM: 0.75 sec) and small events (~16% of a single end-foot volume). These data imply that astrocytes possess distinct signaling domains and that local signaling is an important component of their processing, with most of the activity remaining highly compartmentalized under normal conditions. Further, combining fast 1-to-3D imaging and several synthetic and genetically encoded Ca^{2+} indicators, we developed imaging conditions allowing us to consistently visualize and study the population of fast and local Ca^{2+} “events” in astrocytic processes, whose existence was historically debated. In fact, these small and fast events represent the vast majority of all Ca^{2+} transients detected in a 3D astrocyte. In IP3R2ko mice, originally described as fully devoid of astrocytic Ca^{2+} events, the fast astrocytic Ca2+ activity was reduced (in both number and amplitude) but not abolished, in line with the more recent view that Ca^{2+} dynamics in astrocytes depend on multiple and mutually interactive Ca^{2+} sources.

Supported by grants: ERC Advanced "Astromnesis" and SNSF 31003A-173124

NOTES

L.4. QUAN FINALITZA EL DESENVOLUPAMENT DEL CERVELL HUMÀ ?

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El desenvolupament del cervell humà equival al desenvolupament de la persona, un fenomen complex i llarg que sabem quan comença, però no massa quan acaba. Tradicionalment, la formació del cervell es donava per acabada durant l'adolescència, quan el cos deixa de créixer i on els trets bàsics de la personalitat estan establerts. No obstant, a l'adolescent li queda encara bastant per polir, pel que fa al control de la conducta i les capacitats intel·lectuals. Sospitant que el desenvolupament del cervell es deuria prolongar fins a l'edat adulta, vàrem mesurar canvis de volum al llarg del temps en diverses estructures cerebrals. Els resultats van demostrar que el cos callós augmentava de volum almenys fins els 25 anys. El cos callós és el feix de connexions cerebrals més gran, que comunica els dos hemisferis i fa possible les activitats intel·lectuals més complexes. Així doncs, vàrem descobrir que una estructura representativa de la cognició més elaborada creix fins a l'edat adulta, cosa que avui pot resultar òbvia, però aleshores la idea era bastant revolucionària. L'augment de volum del cos callós correspon a un augment de la mielina dels axons i fa que la transmissió de les senyals entre neurones sigui més ràpida. Posteriorment, en altres estudis, hem fet servir la mielinització com a mesura de la maduració cerebral i hem aprés moltes coses. Per exemple, hem vist com maduren les àrees del llenguatge en nens normals des dels zero fins als tres anys. També hem establert la correspondència entre el retard en la mielinització i el retard mental. Ara sospitem que la ment criminal del psicòpata pot parcialment ser conseqüència d'una mielinització accelerada associada a estrès vital en les primeres fases de la vida. El desenvolupament del cervell humà és un fenomen fascinant i avui disposem de bones eines per estudiar-lo.

NOTES

SESSION 1A

GLIAL CELLS AND INFLAMMATION

O.1. REGIONALLY-SELECTIVE KNOCKDOWN OF ASTROGLIAL GLUTAMATE TRANSPORTERS IN INFRALIMBIC CORTEX INCREASES LOCAL EXCITATORY NEUROTRANSMISSION AND EVOKES A DEPRESSIVE PHENOTYPE IN MICE

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Increases in energy metabolism together with the disturbance of astrocyte number/function in the ventral anterior cingulate cortex (vACC) have been suggested as important contributers to the pathophysiology of major depressive disorder (MDD). Hence, the functional hyperactivity reported in vACC may result from a reduced glutamate clearance from excitatory synapses. Astrocytes are emerging as essential players in synaptic function. Here we examined the functional and behavioral consequences of knocking down GLT-1/GLAST with RNAi strategies in mouse IL cortex under the working hypothesis that a functional hyperactivity in IL may result in a depressive-like phenotype. Unilateral microinfusion of a pool of siRNA sequences targeting GLAST or GLT-1 in mouse IL induced a moderate and long-lasting decrease in their mRNA and protein expression. Intra-IL GLAST/GLT-1 siRNA infusion also reduced GFAP-positive astrocyte density and increased excitatory neurotransmission in layer V pyramidal neurons, as shown by an increased resting membrane potential, increased evoked discharge rate, slow down of evoked EPSC, as well as increased spontaneous EPSC (sEPSC) amplitude and frequency. Moreover, GLAST/GLT-1 knockdown evoked a depressive-like phenotype, as assessed by the FST, TST and SPT, which was reversed by citalopram and ketamine i.p. administration 30 min prior to the test. GLAST or GLT-1 knockdown in IL markedly reduced 5-HT release in the dorsal raphe nucleus and induced an overall reduction of brain derived neurotrophic factor (BDNF) expression in cortical and hippocampal areas of both ipsilateral and contralateral hemispheres. Moreover, Egr-1 labelling suggests that both siRNAs enhance the putative GABAergic tone onto DR 5-HT, leading to an overall decrease of 5-HT function, likely related to the widespread reduction on BDNF expression. These results show that a reduction of glutamate clearance by astrocytes in IL results in marked local and distal changes in neuronal activity, likely associated to the depressive-like phenotype evoked by GLAST/GLT-1 knockdown in IL.

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NOTES

O.2. INTERACTION BETWEEN GPR18 AND CANNABINOID CB2 G-PROTEIN-COUPLED RECEPTORS: RELEVANCE IN NEURODEGENERATIVE PROCESSES

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GPR18, still considered an orphan receptor, may respond to cannabinoids, whose canonical receptors are CB1 and CB2. GPR18 and CB2 receptors share a role in peripheral immune response regulation and are co-expressed in microglia, which are immunological-system competent cells in the CNS. We aimed at determining GPR18 and CB2 receptor expression and function in resting and activated microglia. Receptor interaction was assessed using energy-transfer approaches and receptor activation by determining cAMP levels and ERK1/2 phosphorylation in heterologous cells and primary cultures of microglia from wild-type and Alzheimer's disease (AD) model mice (carry the $APP_{Sw,Ind}$ mutation). In situ heteroreceptor identification was achieved by proximity ligation assays. GPR18 can form heteroreceptor complexes with CB2 but not with CB1 receptors. CB2-GPR18 heteroreceptor complexes displayed particular functional properties (heteromer print) consisting of negative cross-talk (activation of one receptor reduces signaling of the partner receptor) and cross-antagonism (bidirectional blunting of responses mediated by either receptor using selective CB2R or GPR18 antagonists). Evidence of the heteromer print was detected in microglia. Moreover, activated microglia showed increased expression of both CB2R and GPR18, and of heteroreceptor complexes. Due to the important role of CB2R in neuroprotection, we further investigated the heteroreceptors occurrence in primary microglia of $APP_{Sw,Ind}$ transgenic mice. Microglial cells from transgenic but not from wild-type animals showed expression and functional interactions between CB2R and GPR18 that were similar to the activated microglial phenotype. Our results show that GPR18 and its heteromers may play important roles in neurodegenerative processes.

This research was supported by grants (SAF2012-39875-C02-01 and SAF2016-80027-R) from the Spanish Ministry of Economy and Competitiveness (MINECO; grants may include EU FEDER funds) and from the Fundació La Marató de TV3 (grant number 201413330). Financial support by the Polish National Science Center DEC. 2013/11/B/NZ7/04865 is also acknowledged.

NOTES

O.3. P2RY12 INHIBITION IN MICROGLIA REDUCES THE PHAGOCYTOSIS OF NEURONS AFTER AN EXCITOTOXIC INJURY

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Microglia activity in healthy and neuropathological conditions influence neuronal survival and function. In the present study, we investigated whether the blockade of the *G protein*-coupled *purinergic receptor* P2Y12 (P2RY12) modulates microglia activity. P2RY12 is expressed on the surface of platelets and many antithrombotic agents are P2RY12 inhibitors. Recent studies demonstrate that some P2RY12 effects are unrelated to anti-aggregation. In the brain, P2RY12 is selectively expressed in microglia, and apart from its role in migration after a cerebral lesion and myelin phagocytosis after spinal cord injury, its role in microglia is largely unknown. Animals deficient in P2RY12 are protected against ischemic injury (Webster 2013, Gelosa 2014,Yamauchi 2017). To shed light on P2RY12 relevance in microglia in brain pathology, we used a model of excitotoxicity in mixed neuron/glia cultures to study the behavior of microglia in the presence of the specific inhibitor of P2RY12 PSB-0739. Live imaging was performed in cortical neuron/glia cultures prepared from embryos resulting from the crossing between ROSA-CAG-tdTomato flox/flox and CX3CR1-CRE-^{ERT2} mice. In these cultures, microglia express tdTomato after addition of 4-OH-tamoxifen and neuron debris were stained with SYTOX green. Time-lapse images were acquired in a Leica SP5 2-photon confocal microscope and we measured motility parameters, phagocytosis of neuron debris and neuronal death with ImageJ and macros we developed. The results show that P2RY12 inhibition reduced excitotoxicity-induced phagocytosis and neurites damage. We are currently investigating whether inhibition of phagocytosis by P2RY12 blockade affects neuronal survival.

References: Webster 2013 PloSOne 8:e70927; Gelosa 2014 JCBFM 34:979; Yamauchi 2017 Sci Rep 7: 12088

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NOTES

O.4. CCR2+ MONOCYTES PROMOTE RECOVERY FOLLOWING STROKE IN MICE

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Questions: Classically, infiltrating macrophages derived from monocytes were regarded as detrimental for the ischemic tissue, but current evidence is challenging this view. The chemokine receptor CCR2 is critical for monocyte infiltration and CCR2-deficient mice show smaller lesions and better functional outcome after ischemic stroke. However, several studies reported exacerbated ischemic lesions and functional outcome after blocking CCR2. Globally targeting CCR2 affects various cells expressing CCR2. The objective of this study was to investigate the contribution of monocytes to the ischemic lesion by cell-specific CCR2 deletion.

Methods: We generated mice lacking CCR2 in myeloid cells crossing LysM-cre+ mice with CCR2flox/flox mice (donated by Dr. Manolis Pasparakis). Permanent ischemia was induced in adult $CCR2^{flox/flox}$cre- (monocyte $CCR2^{+/+}$) and $CCR2^{flox/flox}$cre+ (monocyte $CCR2^{-/-}$) mice of both sexes. Brain immune cell populations and gene expression was studied by flow cytometry and qRT-PCR, respectively, at days 1, 4, and 15 postischemia. We studied the brain lesion by T2w-MRI and assessed functional outcome with behavioral tests.

Results: Monocyte CCR2-deficiency reduced brain infiltration of $Ly6C^{hi}$ and $Ly6C^{lo}$ monocytes, without affecting neutrophil infiltration. Infarct volume was similar in both genotypes. However, mice with CCR2-deficient monocytes showed a lower expression of pro-inflammatory genes, IL-1b, TNFα, COX2, MMP3, at 24h compared to mice with $CCR2^{+/+}$ monocytes. The neurological function in the rotarod and pole tests showed spontaneous improvement from 1 to 15 days after stroke in mice with $CCR2^{+/+}$ monocytes, but not in mice with CCR2-deficient monocytes.

Conclusions: $CCR2^{+}$ monocyte infiltration induces a pro-inflammatory profile within the first day after stroke that seems to be necessary to support spontaneous recovery of the neurological function. The study suggests that monocytes mediate inflammatory responses in acute stroke that are required for secondary resolution and repair.

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NOTES

O.5. CLASSIFICATION OF MICROGLIAL CELLS BASED ON MULTIPLE PARAMETERS MEASUREMENTS: A LIVE IMAGING STUDY

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Microglial are the immune cells of the brain. They are constantly surveying the environment to detect any presence of pathogens to destroy them. In physiological conditions, microglia get rid of dysfunctional synapses (pruning) and neuronal molecules bind to microglia receptors to maintain the latter in a harmless state. However, after a brain damage, the balance can be disrupted. Under pathological situations such as neurodegeneration or stroke, damage-associated molecular pattern molecules (DAMPs) are released by injured neurons or astrocytes and activate microglia. Depending on the environment, microglia promote the regeneration of the tissue or trigger inflammation and kill neurons. Recent studies identified a sub-population of "neuroprotective" microglia among activated microglia in animal models of neurodegenerative diseases. However, the identification of different populations in a cell culture can be very challenging. Features such as morphology or phagocytic activity of microglia have been described as indicators of microglia activation and function. Softwares are available to perform tracking and morphometric analysis. However, they are often not sophisticated enough to segmentate overcrossing cells and quantifications are usually very time-consuming. We set up a live cell imaging model of neuron/glia co-culture challenged with excitotoxicity, the main cause of neuronal death in the ischemic brain, and monitored parameters reflecting microglia behavior in physiological and pathological conditions. Using time-lapse obtained from an automatized confocal microscope, we have been developing macros for Image J to segmentate microglia, quantify cell motility (velocity, displacement, chemotaxis), shape and phagocytosis. Our final goal is to provide a package of automated programs measuring multiple parameters to classify microglia by morphology, motility and phagocytic activity and to try to identify a sub-class of microglia with a neuroprotective profile. Here, we are presenting the results of the quantification of microglia morphology and motility obtained with the macros we designed.

This project is funded by a MINECO grant (SAF2017-87459-R) and the European Union's Horizon 2020 research and innovation program under grant agreement No 654248. AO is the recipient of a MINECO FPI fellowship

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O.6. GUT BRAIN-AXIS IN STROKE: INTESTINAL IMMUNE SYSTEM IS ALTERED BY SURGICAL STRESS IN THE MOUSE MODEL OF CEREBRAL ISCHEMIA/REPERFUSION

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The nervous system is functionally and anatomically connected to the immune system. Acute stroke induce neuro-hormonal responses affecting the immune system. Several lines support stroke-induced alterations of gut immune cells that in turn exacerbate the brain inflammatory response. Experimental stroke models require surgery and deep anesthesia that do not take place in stroke patients. Both anesthetic and surgical stress affects systemic inflammation and leukocyte populations in the bone marrow. The objective of this study was to identify stroke-induced alterations in gut immune cells by specifically monitoring the changes induced by surgery and anesthesia in appropriate sham conditions. We induced ischemia in adult male mice on the C57BL/6J background by 45-min middle cerebral artery occlusion (filament model) and 48 h reperfusion. To control for the effects of experimental surgery and anesthesia (90 min in total) we conducted in parallel sham-operations, where we followed all the procedures excepting placement of the filament. We also studied control mice devoid of any intervention. Dissection of the gut was performed and intestinal immune cells were quantified by flow cytometry. Cell analysis showed a drastic reduction of IEL cell number (B and T cells) in small intestine after stroke vs. controls. However, the main causative mechanism was related to surgical stress since the same response was found in sham-operated mice. Treg cells showed particular changes in the SI where their number decrease in IEL and have a tendency to increase in LP in response to surgical stress. As a conclusion, models of experimental stroke introduce artifactual alterations of the gut immune system due to anesthesia and surgical stress that are not mediated by specific immune response to stroke. Improved methodologies and/or strict control of non-stroke related technical aspects are critical to improve the success of stroke translational studies.

NOTES

SESSION 1B

NEURODEVELOPMENT AND STEM CELLS

O.7. STUDYING AXON GUIDANCE AND NEURONAL MIGRATION WITH SELECTIVE INTERFERENCE OF SECOND MESSENGER FUNCTION *IN VIVO*

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Second messengers are mid-point relays in signaling cascades governing a wide range of cellular functions. Their precise control is required at several steps leading to the sound connectivity of the nervous system. How neurons are able to discriminate between second messenger signals arising from different stimuli is unknown. Thus, being able to interfere with transient or local changes in second messenger concentration in a developing neuron is critical to unveil the developmental mechanisms wiring the brain. We have developed "*SponGee*" and "*SpiCee*", a pair of genetically-encoded buffers that alter physiological changes in the concentration of cGMP and calcium respectively. These tools enable disrupting signaling cascades with cellular and subcellular resolution and might shed light on how neurons perceive the significance of second messenger variations in response to guidance stimuli. They provide a new specific approach to investigate how nervous networks arise. We provide evidence that *SponGee* and *SpiCee*, both in soluble form or targeted to the lipid raft or non-lipid raft microdomains of the plasma membrane, are able to buffer changes in the respective second messenger concentration. Using in utero electroporation we show that calcium and cGMP signaling are crucial for the correct migration and placement of newly generated cortical neurons. We further show how second messenger signaling in lipid rafts is required for the response of retinal ganglion cell axons to guidance cues.

NOTES

O.8. IDENTIFICATION OF STRIATAL PROGENITOR SUB-POPULATIONS DURING HUMAN EMBRYONIC STEM CELL DIFFERENTIATION USING SINGLE-CELL RNASeq

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Cell therapy is a key strategy in regenerative medicine to replace damaged tissues, and a viable approach for treating Huntington's disease (HD) where striatal GABAergic medium spiny neuron (MSN) degeneration occurs. Implementation of cell replacement strategies requires the development and characterization of human pluripotent stem cell *in vitro* differentiation protocols that efficiently generate the striatal progenitors required for transplantation. Previously we have successfully transplanted hPSC-derived neural progenitors into mouse striatum. However we are aware that various different neural progenitor sub-types are present within the transplanted cell population at this stage and in the future aim to transplant solely striatal progenitors. To identify the neural progenitor sub-types that are present at the transplantation timepoint, and specific cell surface markers for those cell types, we performed massively parallel RNA single-cell sequencing (MARS-seq) on 3 human embryonic stem cell lines *in vitro* differentiated to the transplantation timepoint. The transcriptomes of 4378 cells were obtained with both high efficiency (96%) and technical reproducibility. Bioinformatic analysis using bigSCale, a novel and highly effective analytical framework for single-cell data, identified 2 main populations of neural precursors cells (NPCs) and neuroblasts (NBs). A third small population was identified which consists of transient intermediate cells that were captured during the process of differentiating from a NPC to a NB. Further analysis identified specific cell surface markers for these 3 populations. Work is currently ongoing to identify the specific sub-types that are present within the NPC and NB populations. We anticipate that using this data we will improve the efficiency of our *in vitro* differentiation protocol. Furthermore we will be able to purify specific striatal progenitor cell types from *in vitro* differentiated cultures to produce a homogeneous population for transplant which will improve HD cell therapy efficacy.

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

NOTES

O.9. MODELING TYROSINE HYDROXYLASE DEFICIENCY USING INDUCED PLURIPOTENT STEM CELLS

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Tyrosine Hydroxylase deficiency (THD) is characterized by a defect in the Tyrosine Hydroxylase (TH), the enzyme that catalyzes the rate-limiting step in the biosynthesis of dopamine. This pathology has variable degrees of dystonia, parkinsonism and intellectual disability. Two clinical phenotypes have been described; the THD “B” phenotype produces a severe encephalopathy of early-onset with sub-optimal L-Dopa response, whereas the “A” phenotype has a better L-Dopa response and outcome. We have generated induced pluripotent stem cell (iPSC) lines from one patient with the severe phenotype (THD-B), one patient with the less severe form (THD-A) and two unrelated healthy individuals (Ctrl). We used non-integrative episomal vectors to generate 2-4 independent iPSC lines per individual, that were thoroughly characterized and shown to be fully reprogrammed to pluripotency. To promote DA neuronal differentiation, we used a three-stages protocol involving: (1) hESC aggregation (via EB formation), (2) neural induction and (3) DA neuron maturation. When we analyzed the neuronal cultures obtained from either THD-B and THD-A-iPSC, we found a reduction of DAn by 63% compared to Ctrl DAn. This data was confirmed by WB and HPLC studies that revealed lower levels of TH expression and L-DOPA in DAn derived from THD patients, but not in Ctrl DAn. We are currently developing isogenic iPSCs by using CRISPR/CAS9 technology to correct the mutation, that will be assayed together with their original mutated counterparts in parallel, for comparison and rescue of the pathological phenotype.In a second approach, we are generating GABAergic and Glutamatergic neurons, from THD-iPSC, to assess the amount of GABA+ neurons, neuron’s maturity and arborization, and the synaptic density compared to Ctrl neurons. The availability of THD patient-specific iPSC lines, as well as isogenic lines, will hopefully shed light on potential mechanisms contributing to THD pathogenesis and lead to new treatments.

NOTES

O.10. HUMAN iPSC-MOUSE CHIMERAS TO STUDY HUNTINGTON'S DISEASE PHENOTYPES

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Huntington's disease (HD) is a hereditary neurodegenerative disorder mainly characterized by striatal atrophy and degeneration of medium spiny neurons (MSNs). Although mouse models have provided a substantial amount of information about HD, they show important limitations for understanding the pathogenesis in humans. Current *in vitro* HD human models can recapitulate some disease phenotypes, but they are not useful for studying long-term differentiation, aging and the establishment of brain connections. Interestingly, new *in vivo* chimeric models using HD patient-derived induced pluripotent stem cells (iPSCs) transplanted into mice could avoid these shortcomings, by allowing cell differentiation and aging within a physiologically relevant environment. We have transplanted iPSC-derived telencephalic progenitors from healthy subjects and HD patients into the mouse developing forebrain, at both embryonic and neonatal stages. At these early ages, patterning cues present in the host developing brain act to instruct specific cell fates and play a key role in determining the migration, connectivity and functional integration of engrafted cells. Following transplantation into the mouse neonatal striatum, the vast majority of cells express CTIP2, including a subpopulation co-expressing DARPP-32, indicative of MSN identity. Furthermore, human iPSC-derived differentiated neurons sent axons towards MSN targets and were able to establish synapses, suggesting functional integration within the basal ganglia circuitry. Remarkably, transplanted cells survived up to 5 months and HD neurons recapitulated human HD pathology, as evidenced by mutant huntingtin aggregation and striatal degeneration. Preliminary results from *in utero* transplantation into the lateral ventricles of E14.5 embryos, show a wider dispersion of grafted cells compared to neonatal transplantation. Moreover, HD cells display increased branching one month after grafting. In summary, we conclude that human iPSC-mouse chimeras are a useful tool for modeling human HD *in vivo*.

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NOTES

O.11. NEURODEVELOPMENTAL ALTERATIONS IN HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is a fatal neurodegenerative disorder that manifests itself through motor and cognitive symptoms due to a predominant loss of Medium Spiny Neurons (MSNs) in the striatum. HD research has focused on characterizing the pathogenesis during the adulthood. However, growing evidences suggest that developmental alterations may occur and could be a key factor in HD. These subtle alterations may determine the future vulnerability of certain cell types, such as MSNs of the striatum. This work aims to study if there are neurodevelopmental alterations in the HD mouse model, Q175. We isolate both mantle and germinal striatal zones of E16.5 from WT and HD embryos by laser micro-capture and we then compared its gene expression by bulk RNA-seq analyses. The results show significant differences in the gene expression patterns in the mantle zone of HD embryos compared to controls. The observed alterations suggest defects in neuronal generation, neuronal growth and maturation, as well as alterations in cellular processes such as mitochondrial metabolism, vesicular transport and calcium homeostasis, all of them reported as damaged pathways in HD patients. All together, these results indicate that HD pathogenesis starts early in neurodevelopment, thus opening a new promising therapeutic window for HD.

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NOTES

O.12. DECIPHERING NMDAR MUTATIONS PATHOGENICITY IN NEURODEVELOPMENTAL CONDITIONS

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Introduction: NMDA ionotropic glutamate receptors play pivotal roles in synaptic development, plasticity, neural survival, and cognition. Recent advances on Next-Generation Sequencing have provided valuable identification of de novo mutations affecting the genes coding for NMDAR subunits that might affect protein stability, surface trafficking and/or biophysical properties, leading to neurodevelopmental and psychiatric disorders.

Hypothesis: We hypothesize that NMDAR de novo mutations can be functionally grouped and stratified, for further design of personalized therapies.

Objectives: In this project, we aim to evaluate the potential pathogenicity of NMDAR subunit variations associated with neurological conditions and to develop personalized therapies.

Results: We have recruited paediatric patients from European countries and completed the predicted functional mutations groups with genetic variants reported in the databases. Site-directed mutagenesis was performed towards the introduction of the mutations in HA-GluN1 or GFP-GluN2B subunits and further transient transfection in cell lines. Biochemical analysis was performed in HEK293T cell extracts, showing differences in protein stability of particular mutant NMDARs. Immunofluorescence analysis of transfected COS-7 cells was performed and allowed the identification of critical amino acids affecting surface trafficking of mutant NMDARs. In summary, our preliminary results are defining different categories of NMDAR de novo mutations. Further biochemical, cellular and functional studies are required to complete this preliminary classification of de novo NMDAR subunit mutations.

NOTES

SESSION 2A

NEURODEGENERATIVE DISEASES I

O.13. PRESENILIN REGULATES TAU PHOSPHORYLATION AND INFLAMMATION DURING NEURODEGENERATION

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Alzheimer's disease (AD), the most common cause of dementia, is characterized by gradual loss of cognitive abilities, especially memory. AD main pathological features include amyloid-β deposition and hyperphosphorylated tau protein accumulated in neurofibrillary tangles (NFT) in addition to neuronal loss, neuroinflammation and autophagic dysfunction. Dominantly inherited mutations in the Presenilin (PS) genes, the catalytic subunit of γ-secretase complex responsible for amyloid-β generation, are the major cause of familial AD (FAD). Moreover, PS mutations are linked with frontotemporal dementia (FTD), which is characterized by accumulation of aggregated phosphorylated tau. At present, the molecular mechanisms by which PS mutations lead to tau phosphorylation are largely unclear. Interestingly, loss of PS function in brain-specific PS1/PS2 (PS) conditional double knockout (cDKO) mice results in increased cerebral tau phosphorylation, neurodegeneration and inflammation. In this work, we studied tau pathology and its relationship with astrocytic and microglial activation, autophagic function and memory in control and PS cDKO mice during aging. Our results show an age-dependent tau phosphorylation in the cortex and hippocampus of PS cDKO mice associated with increased p25/Cdk5 levels, not only in neurons, but also in astrocytes, microglia and oligodendrocytes. This phenotype is associated with increased autophagic, microglial and astrocytic markers. Interestingly, inactivation of PS in human tau transgenic mice results in altered tau pathology and exacerbated memory impairments. Taken together, these results provide evidence that loss of PS function in neurons leads to tau hyperphosphorylation, autophagic dysfunction, brain inflammation and memory loss during neurodegeneration

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NOTES

O.14. NEUROPROTECTIVE MECHANISMS OF RESVERATROL IN A HIGH-FAT DIET-FED ALZHEIMER'S DISEASE MOUSE MODEL

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Over the last decades there has been an over consumption of foods rich in animal fats and refined carbohydrates that has led to an obesity pandemic. Obesity increases the risk of many chronic diseases including Alzheimer's disease (AD). Nevertheless, reducing caloric intake has showed to delay age-related diseases and the natural compound resveratrol has emerged as a potent mimetic of this caloric restriction. The mechanisms underlying both effects are not fully understood and, as potential therapeutic targets, need to be thoroughly analyzed. To this end, we have analyzed molecular pathways involved in memory loss in mice as a result of a high-fat diet (HFD) administration and the role that resveratrol supplementation on this diet may play. We have focused on 6-months-old 5XFAD mice, which are transgenic AD mice with high amyloid burden, and wild type (WT) mice. Both mice strains fed with HFD showed impairment of recognition memory but when resveratrol was added to the HFD, recognition memory was restored. Moreover, our results indicate that HFD boosts amyloid pathology in both, WT and 5XFAD mice, mainly through the potentiation of the amyloidogenic pathway and the mitigation of the non-amyloidogenic one. Namely, we identified an increase in β-secretase 1 protein levels in the cerebral cortex of both strains after HFD administration. Tau pathology was as well aggravated in both strains, showing increased levels of hyperphosphorylated tau. Interestingly, HFD altered the functionality of the ubiquitin-proteasome system, being the most significant changes the induction of proteasome caspase-like and chymotrypsin-like activities that could not prevent the higher accumulation of ubiquitin-tagged proteins for degradation. Remarkably, resveratrol demonstrated the capability to normalize all the pathological changes induced by the HFD in both WT and 5XFAD mice, confirming its preventive and therapeutic potential against neurodegeneration.

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NOTES

O.15. RNA-Seq DIFFERENTIAL EXPRESSION ANALYSIS OF DENDRITIC CELLS AND MICROGLIA IN THE ISCHEMIC BRAIN TISSUE OF MICE

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Seminal studies reported the presence of antigen-presenting cells in the ischemic brain and identified some of these as infiltrating dendritic cells (DCs). However, microglia can also present antigen and both phenotypic and functional differences between these cell populations are not clear. To investigate the differential functions of DCs and microglia in the injured brain tissue, we compared their gene expression profile in the ischemic brain.

We induced transient ischemia in C57BL/6J adult mice and studied the brain tissue 4 days later. We FACS sorted eYFP$^+$ cells from DC reporter mice expressing eYFP under the CD11c promoter. We also FACS sorted microglia from control and ischemic brains of reporter mice that produce fluorescent CX3CR1$^+$ microglia. RNA was extracted from the sorted cells and analysed by RNA-Seq. We specifically identified infiltrating DCs by generating parabiotic mice with CD11c-eYFP and littermate wild type (wt) mice. The wt mouse of each parabiotic pair was subjected to ischemia and the brain was obtained 4 days later for analysis.

We identified numerous genes differentially expressed in DCs vs. microglia of the ischemic brain, and we also compared DCs obtained from the brain and the spleen. Genes related to antigen presentation were overrepresented in DCs compared to microglia and genes typically expressed in microglia showed negligible expression in DCs, suggesting that DCs are a distinct population of cells. We observed eYFP$^+$ cells in the ischemic brain tissue, but not in the contralateral hemisphere of the parabiotic wt mice, demonstrating the infiltration of peripheral DCs. We are currently comparing the gene expression profile of eYFP$^+$ cells obtained from the ischemic brain tissue of parabiotic mice to that of CD11c-eYFP mice.

This study shows that DCs infiltrate from the periphery to the ischemic brain tissue and that these cells have a distinctive gene expression signature.

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NOTES

O.16 MOLECULAR OVERVIEW OF AN AMYOTROPHIC LATERAL SCLEROSIS MICE MODEL: AN INSIGHT TO THE BDNF/TRKB SIGNALING PATHWAY AND ITS COUPLED PKCs AND SNARE/SM TARGETS

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Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease characterized by progressive motor weakness. It is classically accepted that it is caused by motoneuron degeneration leading to a decrease in muscle stimulation. However, ALS is being redefined as a distal axonopathy in which neuromuscular junction (NMJ) degeneration precedes and may directly cause motoneuron loss. In the NMJ, several metabotropic receptor-mediated signaling pathways converge on effector kinases that phosphorylate targets related with structural and functional changes that mediate synaptic stability and neurotransmission. We have shown that under physiological conditions, nerve-induced muscle contraction regulates BDNF/TrkB signaling to retrogradely modulate presynaptic PKCs, which are directly involved in neurotransmission. Synaptic dysfunction at the NMJ by the loss of contact may significantly contribute to motor impairment and muscle atrophy in ALS patients. Accordingly, we investigate whether BDNF signalling and its downstream PKCs together with the SNARE/SM proteins of the exocytotic machinery (Munc18-1 and SNAP-25), are altered in the skeletal muscle of symptomatic SOD1-G93A mice.

Results show that most of the analyzed molecules are strongly affected in symptomatic ALS mice plantaris muscles, displaying a very characteristic molecular pattern. Several of the changes seem to be specific and directly involved in the pathogenesis while others can be explained by a fast-to-slow transition during the disease process

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NOTES

O.17 TARGETING JNK1 AS A METHOD TO IMPROVE METABOLIC-DERIVED COGNITIVE DEFICITS

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Nowadays, metabolic alterations have been deemed to be a possible source for the development of sporadic forms of Alzheimer's disease through Type III Diabetes. Back in 1999, similar patterns were described in The Rotterdam Study. Specifically, resistance to insulin signalling would affect negatively physiological neuronal activity, favouring its degeneration. Also, neuroinflammation and cellular stress have significant roles in these mechanisms. The cJUN N-terminal Kinases (JNK) are a subfamily of the Mitogen Activated Protein Kinases, which are response elements to many cellular stimuli. They are expressed from three different genes (Mapk8, Mapk9 and Mapk10) which produce up to 10 different products, that are later classified into three isoforms. Isoform 1 has been described to be a regulator of macrophages, as well as the cytoskeleton and the insulin receptor (IR). Our results showed how lack of JNK1 expression led to reduced basal body weight, along with increased resistance to the effects of high-fat diets (HFD) enriched with palmitic acid. Also, there was increased insulin sensitivity and molecular response of the IR signalling pathway. Behavioural novel object recognition test examination also showed how in JNK1 knock-out mice, there was neuroprotection against diet-induced memory loss and, how complementary analysis detected no alterations in dendritic spine density and morphology. Furthermore, evaluation of cellular stress biomarkers and neuroinflammation-related cellular types, like astrocytes and microglia, allowed for the determination that wild-type animals that only included HFD feeding presented increased patterns of neuroinflammation and cellular stress, while there was a reduction in the animals that had had their JNK1 gene knocked-out when comparing with the controls. In conclusion, JNK1 inhibition has proved to be beneficial in the considered mechanisms and, may prove to be a possible target for a pharmacological approach in the treatment of sporadic neurodegeneration.

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NOTES

O.18 SEVERE CORTICAL AFFECTATION AFTER COMPLEX I SUBUNIT DELETION IN CCK-EXPRESSING CELLS

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Leigh syndrome (LS), the most frequent infantile mitochondrial disease, causes failure to thrive, ataxia and cognitive decline. A wide range of genetic mutations, in the nuclear or mitochondrial genome, can trigger LS and results in most cases in early death.

We generated an animal model for LS lacking a mitochondrial complex I subunit (Ndufs4KO mouse), recapitulating the majority of the clinical signs present in LS patients, such as: ataxia, growth retardation and early lethality. While motor alterations are ubiquitous in LS patients, cortical affectation and cognitive decline are also widely reported. However, the severe phenotype and short lifespan of the Ndufs4KO mice hinders the study of cognitive deficits. Therefore, we set to develop a cortical-centered Ndufs4KO. Given that Cck (cholecystokinin) gene is extensively expressed in the brain cortex, we created an animal lacking Ndufs4 gene in Cck expressing cells (Ndufs4cKOCck mouse). As expected from a more restricted Cre expression, Ndufs4cKOCck mice, in contrast to Ndufs4KO, did not present a reduction in lifespan or body weight. However, they manifested motor coordination deficits and decreased locomotion in an open field. Histopathologically, gliosis and neuronal death was detected in different cortical areas: motor cortex (M1 and M2), somatosensory cortex (S1 and S2), cingulate cortex (Cg1, Cg2, PrL and IL) and visual cortex (V1). Magnetic resonance imaging showed decreased ADC (apparent diffusion coefficient) values in the same brain areas, due to a cytotoxic edema. Furthermore, several other brain areas, such as hippocampus, striatum and thalamus manifested an inflammatory response in the absence of overt lesions. Detailed behavioral phenotyping of these animals is currently underway to establish the relative contribution of each brain area to the pathology, providing novel insight on the cellular and molecular mechanisms leading to cognitive decline in mitochondrial disease.

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NOTES

O.19 ALTERED FUNCTIONAL CONNECTIVITY AND NETWORK DYNAMICS IN HUNTINGTON'S DISEASE STRIATAL PRIMARY CULTURES REVEALES THROUGH LARGE-SCALE CALCIUM IMAGING

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Striatum, the main hub of the basal ganglia circuitry, is the most affected region in Huntington's disease (HD). In HD, mutant-huntingtin (mHtt) causes an excitatory-inhibitory imbalance of the basal ganglia output pathways and induces motor symptoms. Although alterations of striatal Medium-sized Spiny Neurons (MSN) occur at early stages of the disease, little is known about how this translates into functional changes in the network dynamics. Using high-resolution calcium imaging, we recorded simultaneously hundreds of cells from striatal and cortical primary cultures in WT and the R6/1 mouse model of HD and characterized the spontaneous collective activity patterns.

Although striatum is mainly composed by inhibitory neurons, we showed that isolated striatal cultures are functionally active. Moreover, R6/1 striatal primary cultures exhibit less proportion of active neurons respect to WT. Nonetheless, both WT and R6/1 include a subset of neurons that display collective bursting activity, indicating the presence of a functional network. Collective bursts present a duration and amplitude similar between genotypes whilst the interval between bursts is significantly decreased in R6/1 striatal cultures respect to WT. Blockade of GABAA receptors by bicuculline (BIC) increased number of active neurons and boosted coherent activity throughout the culture in both WT and R6/1, however, inter burst interval is increased in WT and remained unaltered in R6/1, equaling both genotypes. These data suggest an impairment in network burst generation in R6/1 striatal neurons. Additionally, functional connectivity analyses revealed decreased input degree in R6/1 striatal network as well as less number of connected modules of neurons, pinpointing a defective communication in R6/1 striatal neuronal network. Therefore, our data suggest that striatal network dysfunction in HD may arise from local inhibition, but further analysis is required to characterize the contribution of different neurotransmitter systems to the striatal network dynamics in healthy and HD cultures.

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NOTES

SESSION 2B

NEUROTRANSMITTER RECEPTORS AND NEUROPHARMACOLOGY I

O.20. DOPAMINE D_2–LIKE AUTORECEPTOR PARTIAL AGONIST ANTIPSYCHOTICS DECREASE DOPAMINE SYNTHESIS

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Psychotic episodes of schizophrenia are characterized by elevated presynaptic dopamine synthesis, availability and release that may occur even in the absence of external stimuli[1]. Dopamine D_2–like autoreceptor (D2R) can modulate dopaminergic neurotransmission in the presynaptic neuron. The use of D2R partial agonist as antipsychotic drugs has been accepted since aripiprazole was approved by US FDA in 2002. After aripiprazole, the latest approved D2R partial agonists antipsychotics was brexpiprazole and cariprazine. Properties of all three D2R partial agonists were examined by monitoring the changes of dopamine synthesis in rat brain striatum incubated ex vivo. We report that the activation of D2R by partial agonists inhibited the synthesis of dopamine in presynaptic neurons dose-dependently. The efficacy of all three partial agonists was compared, with the most potent being aripiprazole > cariprazine > brexpiprazole. The novel characteristic of D2R partial agonists is being “dopamine stabilizers” meaning the drug can stimulate or block dopamine receptor depending on endogenous dopamine and therefore maintain an intermediate level of stimulation. The effect of dopaminergic tone on the properties of the drugs was tested by comparing a basal condition (2mM K^+, low dopaminergic tone) and a stimulated condition (15mM K^+, where dopamine release mimics the extracellular conditions of depolarized dopaminergic terminal). Taken together, the results show that all dopamine D2–like autoreceptor partial agonist approved as antipsychotics decrease presynaptic dopaminergic synthesis,and may be interesting to correlate this effect with their therapeutic efficacy as antipsychotic drugs.

[1] Howes O.D. et al. The Nature of Dopamine Dysfunction in Schizophrenia and What This Means for Treatment. Arch. Gen. Psychiatry 69, 1–11 (2012).

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NOTES

O.21. *IN VIVO* PHOTOMODULATION OF GABA AND GLYCINE RECEPTOR CHANNELS

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Neuronal networks are highly complex interactions, which determine even the finest behaviour. The major inhibitory pathways in the central nervous system (CNS) act through chloride ion flux, which are mostly driven by fast-acting ionotropic GABAA and Glycine receptors (GlyR). All of them share structural similarities and belong to pentameric ligand-gated ion channels of the Cys-loop family. Photopharmacology has proven to be advantageous for spatial and temporal control of biological processes without interfering the system natural dynamics and outcomes. Physiology can be tuned with photomimetic ligands and naturally occurring complex network responses can be segmented into light dependent activities discerning relevant data from a vast matrix of results. Zebrafish (Danio rerio) larvae constitute an excellent animal model for studying and screening photoswitchable molecules in vivo. The use of up to 96 animals simultaneously allows a parallel high throughput data recovery system to analyse high complexity movements and behaviours, all of it with the use of photopharmacology. Here, we aimed at introducing an effective and reliable methodology for high throughput screening of photoswitchable compounds from specific neuronal correlated diseases up to possible toxicological outcomes. We focused on the study of the main inhibitory neuronal pathways and their locomotion outcomes on a reliable and comparable animal model. Larvae zebrafish treated with UR-DW290 maintained higher activity in terms of swimming distance (mm) during the relaxation period and UV-Blue light cycles in comparison to controls. We propose the combination of high throughput screening and optopharmacology tools for the study and characterisation of zebrafish larvae behaviour focusing on their swimming activity. We identified a first photoswitchable molecule for glycine receptor modulation in vitro and in vivo, UR-DW290, which increases basal activity in zebrafish larvae. This increase is tuneable with UV and Blue light illumniation.

NOTES

O.22. PHOTOSWITCHABLE DYNASORE ANALOGS TO CONTROL ENDOCYTOSIS WITH LIGHT

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The spatiotemporal control of cellular dynamic processes has great fundamental interest but lacks versatile molecular tools. Dynamin is a key protein in endocytosis and an appealing target to manipulate cell trafficking using patterns of light. We have developed the first photoswitchable small-molecule inhibitors of endocytosis, by a stepwise design of the photochromic and pharmacological properties of dynasore, a dynamin inhibitor. We characterized their photochromism with UV-visible and transient absorption spectroscopy and their biological activity with transferrin uptake assays in live cells using confocal microscopy and flow cytometry. They are water-soluble, cell-permeable, and photostable, and enable fast, single-wavelength photoswitchable inhibition of clathrin-mediated endocytosis at micromolar concentration.

NOTES

O.23. EFFECT OF PRIDOPIDINE, A DOPAMINE STABILIZER, ON THE PHENCYCLIDINE-BASED ANIMAL MODEL OF SCHIZOPHRENIA

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Schizophrenia is a neuropsychiatric disorder with unknown etiology. It is characterized by a simultaneous deregulation of the dopaminergic and glutamatergic systems, so its pharmacotherapy mostly relies on restoring dysregulated striatal dopamine and prefrontal cortex glutamate neurotransmission. The compounds acting on the dopaminergic system are predominantly antagonist of dopamine D_2 receptors (D_2Rs), so they reduce the hyperdopaminergic state on the striatum of schizophrenic patients, but they have important prejudice concerning motor side effects. Dopamine stabilizers could solve this problem, since they stabilize these receptors rather than block them harshly. ACR16 or pridopidine is a well-known dopamine stabilizer which was firstly synthetized as a D_2R ligand but lately it was described to have higher affinity for sigma 1 receptor [1]. This drug is currently on clinical trials for Huntington disease [2], but its effect on psychiatric diseases has not been deeply described yet. Our aim was to study the effect of this dopamine stabilizer on a schizophrenia mouse model and characterize it behaviorally (i.e. cognitive and positive symptomatology). First, memory impairment was induced in mice by administration of phencyclidine (PCP, 10 mg/kg/day) for 10 days, followed by 14 days' treatment with pridopidine (6 mg/kg/day), or saline. Interestingly, mice receiving PCP and saline exhibited deficits in novel object recognition, as expected, while pridopidine treatment counteracted PCP-induced memory impairment. Next, we evaluated the positive symptoms, thus whereas inhibition of spontaneous and phencyclidine-induced locomotion was observed, neither locomotor stimulation in habituated mice, nor any effects on pre-pulse inhibition were detected upon pridopidine treatment. Overall, our results suggested that pridopidine exerts anti-amnesic and potentially neuroprotective actions, thus providing new insights into the therapeutic potential of pridopidine as a pro-cognitive drug.

NOTES

O.24 CHRONIC ADENOSINE A2A RECEPTOR BLOCKADE INDUCES LOCOMOTOR SENSITIZATION AND POTENTIATES STRIATAL LTD IN GPR37 DEFICIENT MICE

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Adenosine A2A receptors ($A_{2A}R$) play a key role modulating dopamine-dependent locomotor activity, as heralded by the sensitization of locomotor activity upon chronic $A_{2A}R$ blockade, which is associated with elevated dopamine concentrations and altered corticostriatal synaptic plasticity. Since the orphan receptor GPR37 modulates $A_{2A}R$ function in vivo, we now tested if the $A_{2A}R$-mediated sensitization of locomotor activity is GPR37-dependent and if this involves synaptic plasticity adaptations. To this end, we administered a selective A2AR antagonist, SCH58261 (1 mg/kg, i.p.), daily for 14 days and the locomotor sensitization, striatum-dependent cued learning and synaptic plasticity (i.e. long-term depression, LTD) were compared in wild-type and GPR37-/- mice. Notably, GPR37 deletion promoted $A_{2A}R$-mediated locomotor sensitization but not striatum-dependent cued learning upon chronic SCH58261 treatment of mice. Furthermore, while acute ex vivo SCH58261 treatment failed to modify striatal LTD, chronic $A_{2A}R$ blockade potentiates LTD in corticostriatal synapses of GPR37-/- mice. Overall, these results revealed the importance of GPR37 regulating basal ganglia circuitry to affect $A_{2A}R$-dependent locomotor sensitization and synaptic plasticity.

Keywords. GPR37, behavioral sensitization, LTD, adenosine A2A receptor

NOTES

O.25. REGULATION OF AMPAR TRAFFICKING BY CPT1C DEPALMITOYLATION

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In the central nervous system, the trafficking of AMPA receptors (AMPAR) is finely regulated by a large number of intracellular proteins binding to AMPAR subunits. Amongst these interacting proteins it has been recently described that carnitine palmitoyltransferase 1C (CPT1C) binding to GluA1 subunit of AMPARs enhances surface expression of these ionotropic glutamate receptors in such a way that synaptic AMPAR content in CPT1C deficient animals is diminished. However, the molecular mechanisms of such modulation remain unknown although a putative role of CPT1C in depalmitoylating GluA1 has been hypothesized. Here, we explore that possibility and show that CPT1C effect on AMPARs is likely due to changes in the palmitoylation state of GluA1. Based on in silico analysis, Ser 252, His 470 and Asp 474 are predicted to be the catalytic triad responsible for CPT1C palmitoyl thioesterase (PTE) activity. When these residues are mutated or when PTE activity is inhibited, the CPT1C effect on AMPAR trafficking is lost, validating the CPT1C catalytic triad as being responsible for PTE activity on AMPAR. Moreover, the histidine residue (His 470) of CPT1C is crucial for the increase in GluA1 surface expression in neurons and the H470A mutation impairs the depalmitoylating catalytic activity of CPT1C. Finally, CPT1C effect seems to be specific for this CPT1 isoform and it takes place solely at endoplasmic reticulum (ER).

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NOTES

SESSION 3A

NEURONAL CIRCUITS AND PLASTICITY

O.26. CARNITINE PALMITOYLTRANSFERASE-1 DELETION IN AGRP NEURONS INCREASES ENERGY EXPENDITURE BY ENHANCING BROWN ADIPOSE TISSUE ACTIVITY

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Food intake and whole-body energy balance are regulated by the brain through a sophisticated neuronal network located mostly in the hypothalamus. In particular, the hypothalamic arcuate nucleus (ARC) is a fundamental sensor for the hormones and nutrients that inform about the energy state of the organism. The ARC contains two populations of neurons with opposite functions: anorexigenic proopiomelanocortin (POMC)-expressing neurons and orexigenic Agouti-related protein (AgRP)-expressing neurons. Activation of AgRP neurons leads to an increase in food intake and a decrease in energy expenditure. It has been suggested that lipid metabolism in the ARC plays an important role in the central control of whole-body energy balance. Yet it is unclear whether lipid metabolism regulates the activity of AgRP neurons specifically. To answer this question, we studied mutant mice lacking carnitine palmitoyltransferase 1A (CPT1A) specifically in AgRP neurons. CPT1A regulates the rate-limiting step in the mitochondrial oxidation of fatty acids (FAs) and therefore plays a central role in the metabolism of lipids. The results of our research demonstrate that the deletion of Cpt1a in AgRP neurons: 1) reduces body weight without affecting food consumption. 2) increases energy expenditure and the activity of brown adipose tissue (BAT). 3) decreases white and brown adiposity and increases the expression of genes involved in lipolysis, such as Atgl, in BAT. Altogether, our results suggest that CPT1A and FAs oxidation in AgRP neurons impact peripheral energy balance without affecting food intake and highlight this pathway as a possible target for therapeutic strategies to decrease body weight.

Founded by: MINECO SAF2017-83813-C3-1-R, MARATÓ TV3 and CIBEROBN

NOTES

O.27. OPTOGENETIC STIMULATION OF CORTICOSTRIATAL PATHWAY AMELIORATES MOTOR BEHAVIOR IN HUNTINGTON'S DISEASE

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The disruption of the basal ganglia network is a key factor for the movement alterations found in Huntington's Disease (HD). HD is a rare and devastating inherited neurological disorder with late-onset manifestations including involuntary movements, psychiatric and cognitive symptoms. Brain HD pathology is most prominent in the striatum, with a selective degeneration of GABAergic medium spiny neurons, responsible of the choreic movements that plague HD patients.

The cortex is the main afference to the striatum. Cortical pyramidal neurons are affected in HD, making the corticostriatal pathway a keystone in the pathophysiology of the neurodegenerative process. Indeed, the debilitating choreic movements of HD patients have been attributed to striatal degeneration induced by the loss of cortically supplied BDNF. In general, there is a progressive disconnection between cortex and striatum manifested as a reduction in spontaneous synaptic activity. Here, we investigate the behavioral consequences of repeated optogenetic cortico-striatal stimulation in WT and R6/1 HD mouse model at symptomatic stages (20wk). We bilaterally injected an AVV-CamKII-ChR2 virus vector, or CamKII-YFP as control, in the motor cortex of 16 week WT and R6/1 mice. We also implanted bilateral fiberoptic cannulas in the striatum. After 4 week recovery, we evaluated changes in motor behavior during repeated optogenetic costicostriatal stimulation. Our results showed that 10 Hz corticostriatal stimulation in R6/1-ChR6 mice induces long-lasting changes leading to improvements in motor learning (accelerating rotarod), coordination (balance beam test), exploratory activity (rearings) and stereotypic behavior (grooming), compared to control R6/1-YFP mice and reaching levels similar to WT mice. This would be the first functional demonstration in vivo of an effective optogenetic induced recovery of HD motor symptoms. Further investigation will help to understand the mechanism involved and to design novel therapeutic strategies aiming to restore network dysfunction in HD.

Funded by MINECO, RETICS and Marató TV3

NOTES

O.28. THE MIGATTE NO GOKUI STATE IN MICE IS HAMPERED BY THE HIPPOCAMPUS

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The dissociative brain functions are still poorly understood in neurosciences. Currently, some lines of evidence indicate that several brain regions are able to modulate very specific cognitive functions. For example, the hippocampus is widely known to regulate spatial memory. However, the same brain regions are able to collaborate or compete between them to reach a final outcome on behavior. In our laboratory we have generated a new transgenic mouse model to permanently label activated neural populations in tightly and controled conditions. Thus, we took advantage of this mouse model to map neural activity in the entire brain during the acquisition and retention of a motor skill. Unexpectedly, we observed that a specific neuronal engram in the hippocampal CA1 was the most persistently activated during the entire task. Enhancing or preventing the activity of this engram indicated that the CA1 regulates the levels of motor learning. We also describe by which molecular mechanism is this engram of the CA1 modulating the levels of motor learning.

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NOTES

O.29 THE ROLE OF ZNF804A AND AKT1 GENES AND CANNABIS USE IN PSYCHOSIS RISK: A GENE-ENVIRONMENT INTERACTION STUDY IN A NON-CLINICAL SAMPLE

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Psychotic disorders are severe neuropsychiatric disorders that affect about 3.5% of the population worldwide. Despite its etiological and pathophysiological underpinnings still remain largely undetermined, it is currently accepted that psychotic disorders are complex disorders caused by genetic (e.g. ZNF804A and AKT1), environmental (e.g. cannabis use) factors and their interaction (EU-GEI 2014). We investigated: i) the association of two schizophrenia-associated genes (ZNF804A and AKT1) with psychosis-associated phenotypes, such as schizotypy and cognitive impairments, and ii) whether lifetime cannabis use (and its dose effect) modulated these associations. Our sample comprised 389 Spanish non-clinical subjects (43.1% males, mean age=21.11(2.19)). Schizotypy was evaluated using the three factors of the Schizotypal Personality Questionnaire-Brief (SPQ-B): Cognitive-Perceptual (SPQ-CP), Interpersonal (SPQ-I) and Disorganized. Sustained attention and verbal memory were measured using the Continuous Performance Test-Identical Pairs and the Logical Memory subtest of the Wechsler Memory Scale, respectively. Subjects were classified according to their frequency of cannabis consumption, and dichotomized as users or non-users. The SNP rs1344706 (ZNF804A) and the haplotype rs2494732 - rs1130233 (AKT1) were genotyped. The effect of genetic variants and cannabis use, along with their interaction, on each of the measured phenotypes was tested using linear models. False Discovery Rate (FDR) correction was applied. In relation to ZNF804A, an interaction between the AA genotype and lifetime cannabis use was found in SPQ-CP (β=1.297 p_{FDR}=0.018). This interaction showed a dose–effect pattern: schizotypy scores increased with increasing frequency of cannabis use (sporadic users: β=0.746 p_{FDR}=0.208; monthly users: β=1.688 p_{FDR}=0.091; intense users: β=1.623 p_{FDR}=0.038). In relation to AKT1, individuals with two copies of the risk haplotype (CA) presented better attention scores (β=0.18 SE=0.059 p_{FDR}<0.001). We can conclude that: i) ZNF804A and AKT1 genes are associated with schizotypy and attention, while cannabis does not in our sample; ii) the interaction between ZNF804A and cannabis modulates psychosis' risk.

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NOTES

O.30. POSTNATAL EXPOSURE TO CHLORPYRIFOS DIFFERENTLY AFFECTS ASSOCIATIVE RECOGNITION MEMORY DEPENDING ON SEX AND APOE GENOTYPE

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Chlorpyrifos (CPF) is one of the most commonly used organophosphorus pesticides. Increasing evidence links subtoxic exposure to CPF with detrimental health effects, including neurobehavioral impairments. Special interest has been directed towards exposures taking place during the neurodevelopmental period. Several studies suggested that genetic differences can confer distinct vulnerability to toxic insults. We are particularly interested in the gene coding for apolipoprotein E (apoE), which is not only involved in lipid transport but also considered a risk factor for several diseases. This study was aimed at assessing the neurobehavioral effects of a postnatal exposure to chlorpyrifos and to interrogate the influence of the genotype and sex. For this purpose, apoE3 and apoE4 targeted replacement mice as well as C57BL/6 mice were exposed to either 0 or 1 mg/kg/day of CPF on postnatal days 10-15. Associative recognition memory was tested in both male and female young adults by means of an Object Recognition Test (ORT). A pharmacological challenge was included in order to determine the contribution of the acetylcholine and GABA neurotransmitter systems. Our results show that females presented higher levels of exploration as well as a better associative recognition memory than males. CPF exposure decreased the preference for the novelty and modulated the sensibility to the cholinergic antagonist scopolamine and to the GABAergic agonist alprazolam, mainly in apoE4 mice. Of especial interest is the effect observed with the cholinergic agonist rivastigmine, which ameliorated the discrimination in apoE4 males but worsened the retention in apoE4 females. Overall, our results provide new insight on the effects of postnatal exposure to CPF on associative recognition memory, being these effects strongly modulated by sex and apoE genotype.

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NOTES

O.31. GENETIC VARIABILITY IN NEURAL EXCITABILITY GENES MODULATES COGNITIVE PERFORMANCE AND BRAIN ACTIVITY. A CASE-CONTROL STUDY IN SCHIZOPHRENIA

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Neural excitability has been pointed out as biological mechanism underlying schizophrenia (SZ) aetiology in the latest GWAS (Pardiñas et al. 2018). Variability in neural excitability genes may underlie cognitive deficits and impaired neural function observed in SZ patients (Green and Harvey 2014). Despite Voltage-Gated Channels related-gens, such *CACNA1C* and *KCNH2*, have previously been associated with SZ, few is known about their effect on cognitive and brain activity phenotypes (Ripke et al. 2014, Huffaker et al. 2009, Krung et al. 2010). Our aim was to analyse the role of *CACNA1C* and *KCNH2* in cognitive performance and brain activity during the performance of a working memory task (n-back) in an fMRI protocol. Genetic association analyses (adjusted by sex, age) of two SNPs (*CACNA1C*-rs1006737 and *KCNH2*-rs3800779) were conducted in a sample of 296 SZ patients and 157 healthy controls (HC) with measures of premorbid-IQ (TAP) and memory (WMS). Neuroimaging analyses (adjusted by sex, age, TAP) were conducted in a subsample of 132 SZ patients and 89 HC. Regarding cognition analyses: SZ patients carrying A allele in *CACNA1C* showed lower premorbid-IQ than GG homozygotes (ß=-1.39, p=0.027, R^2=0.03) while, HC carrying A allele in *KCNH2*, with an allelic dose effect, performed better in memory (ß=3.01, p=0.01, R^2=0.06). Concerning neuroimaging analyses: SZ patients with AA genotype in *CACNA1C* presented a deactivation failure in the prefrontal cortex (voxels=912, z=3.88, p=0.0066) and precuneus (voxels=1132, z=3.83, p=0.0016); whereas, AA genotype in *KCNH2* led to a deactivation pattern in the prefrontal cortex (voxels=1107, z=3.37, p=0.0019), caudate, insula and putamen (voxels=895, z=3.76, p=0.0072) and temporo-parietal regions (voxels=1798, z=3.97, p=3.8e-05). Our findings suggest a possible relationship between *CACNA1C* and alterations in the Default Mode Network activity, which has already been associated with SZ (Pomarol-Clotet et al 2010) and, highlight the role of excitability related-genes in the modulation of intermediate phenotypes of SZ.

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NOTES

O.32. THE ROLE OF *NEURITIN* GENE *IN* MODULATING SCHIZOPHRENIA AGE AT ONSET AND BRAIN ACTIVITY DURING A WORKING MEMORY TASK

Almodóvar C[1], Guardiola M[1,2,3], Salgado-Pineda P[1,2], Gallego C[4], Prats C[2,3], Arias B[2,3], Pomarol-Clotet E[1,2], McKenna PJ[1,2], Fatjó-Vilas M[1,2,3]

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Altered synaptic plasticity is described in schizophrenia (SZ) and may be detectable as deficits in cognitive functions (Froudist-Walsh et al. 2017). Such deficits are more prevalent in early onset patients, considered a subgroup with more homogeneous etiological roots (Nicolson et al. 1999). The plasticity gene *NRN1* has been associated with SZ, age at onset and cognitive performance (Chandler et al. 2010; Fatjó-Vilas et al. 2016). We aimed to investigate: i) the association of NRN1 with schizophrenia-spectrum disorders (SZ-SD), exploring its role in age at onset, and ii) the brain functional correlates of *NRN1* sequence variants. Eleven SNPs in *NRN1* were genotyped. Genetic association analyses were developed in: i) a family-based sample of 542 subjects (158 offspring with SZ-SD, 51.9% ≤age18 (EO)); ii) a case-control sample of 393 subjects (145 healthy controls (HC), 248 SZ patients (38.7% EO)). Neuroimaging genetic analyses (adjusted by sex, age and premorbid IQ) were conducted in 104 HC and 113 SZ patients (fMRI protocol while performing the n-back task). The haplotype CG (rs645649-rs582262) was undertransmitted from parents to EO SZ-SD offspring (p=0.011) and the haplotype CT (rs582262-rs3763180) was less frequent in EO patients than in HC (p=0.0258). Patients carrying the CC genotype (rs582262) showed a failure of deactivation of medial prefrontal regions (mPFC) and cingulate cortex [12.55(16.13)] as compared to GC [1.72(11.19)]; z=4.19, p=2.15e-06;] and to GG [-2.55(16.13); z=4.2, p=5.96e-08]. In HC, C allele carriers showed lower deactivation [1.76(9.66)] in the same regions as compared to GG [-10.64(11.00); z=3.71, p=0.0006]. Our results are in line with previous studies describing: i) the association of *NRN1* variability with SZ-SD and EO; ii) impaired deactivation of the Default Mode Network (that includes mPFC) in SZ patients (Pomarol-Clotet et al. 2008); and iii) the role of *NRN1* in modulating mPFC neurons excitability (Yao et al. 2016).

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NOTES

SESSION 3B

NEUROTRANSMITTER RECEPTORS AND NEUROPHARMACOLOGY II

O.33. ROLE OF THE TRANSCRIPTION FACTOR NR4A2 ON GLUTAMATERGIC SYNAPSES IN THE HIPPOCAMPUS

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Long-term potentiation (LTP) and long-term depression (LTD) are cellular events widely believed to underlie learning and memory. Regulation of the AMPA-type ionotropic glutamate receptor (AMPAR) levels at the synapse is crucial to postsynaptic forms of LTP and LTD of excitatory transmission. Several factors, including neurotrophins, modulate exo- and endocytosis of synaptic AMPARs, and also participate in the regulation of their gene expression. We have previously reported that the neurotrophin BDNF is a target gene of the nuclear receptor related protein 1, Nr4a2, an orphan nuclear transcription factor. However, the potential role of Nr4a2 on AMPAR-mediated synaptic function remains unknown. In this study, we tested whether Nr4a2 transcriptional activity participates in the activity-dependent modulation of AMPARs in cultured hippocampal neurons and acute hippocampal slices. We found that Nr4a2 expression is low in basal conditions, but it increases upon synaptic stimulation in mature hippocampal neurons. This increase is dependent on calcium entry through ionotropic glutamate receptors and requires the activation of the CREB/CRTC1 signaling pathway and calcineurin. In addition, Nr4a2 agonists increased both BDNF and the GluA1 subunit levels in cultures, and partially blocked LTD at CA3-CA1 synapses in hippocampal slices, suggesting that Nr4a2 is involved in the activity-dependent modulation of BDNF and AMPAR-GluA1 subunit. Together, our findings indicate that Nr4a2 could play a role in AMPAR plasticity associated to learning and memory events.

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NOTES

O.34. FUNCTIONAL IMPACT OF DYRK1A-MEDIATED PHOSPHORYLATION OF THE NMDA RECEPTOR

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The glutamatergic synapse is the main structure mediating amino acid excitatory neurotransmission. Function and regulation of the N-Methyl D-Aspartate receptor (NMDAR), an ionotropic glutamate receptor family, has been mostly explored in neurons. Physiologically, NMDARs participate in fundamental neuronal functions (neurogenesis, synaptogenesis, plasticity, cognition and survival) while, under pathological conditions and/or challenges, NMDAR dysfunction can trigger neuronal damage and eventually neuronal death with the resulting clinical manifestations. Our group recently described the ability of DYRK1A kinase (protein kinase associated with Down syndrome and Alzheimer's disease) to phosphorylate the serine residue at position 1048, within the GluN2A subunit, which regulates NMDAR surface expression and function. In the present study, we hypothesized that GluN2A(pS1048) phosphorylation event may affect neuronal (dys)function, giving place to neuronal changes potentially affecting cognitive function. Methodologically, we aimed to develop biological tools to decipher the physiological and pathological relevance of this phosphorylation event.

To this end, we have introduced GluN2A variants (wildtype or phosphomimetic, S1048E) into primary neuronal cultures, either by using lentiviral particles or by transient transfection. Importantly, the in vitro paradigm of synaptic plasticity, namely glycine-induced chemical LTP, showed that synaptic activity elicits the phosphorylation of GluN2A at S1048. These experiments have been accompanied by the study of the morphological outcomes (spine density/morphology and and dendritic arborisation) derived from the presence of GluN2A variants. These molecular studies, together with the ongoing behavioural phenotyping of the knockin mouse model KI-Grin2a-S1048D will provide a clear picture of the potential impact of DYRK1A-mediated phosphorylation of NMDAR in synaptopathy conditions.

NOTES

O.35. ALTERATIONS IN GABA A1 RECEPTOR IN RETT SYNDROME: THE NECESSITY OF EARLY GABAERGIC MODULATION AS A THERAPEUTIC STRATEGY

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Rett Syndrome (OMIM #312750) is a neurodevelopment disorder that causes a severe intellectual disability in 1 out of 10.000 children, mostly due to spontaneous mutations in the X-linked gene MeCP2, and is typically characterized by a neurodevelopment regression at 6-18 months of life after a normal early development. Patients experience loss of the acquired skills, such as communication capacities, and a plethora of symptoms including severe autistic features, loss of purposeful use of hands, stereotypes, organic dysfunctions, apnea/ hyperpnoea and seizures.

The lack of therapeutic options for Rett syndrome urges the description of novel treatable targets. Despite its high molecular complexity, growing evidence support an unbalance in glutamatergic/ GABAergic neurotransmission underlying Rett pathophysiology. Accordingly, in this study we aimed to unveil new potential alterations that could constitute actionable targets within these neurotransmission systems.

We analyzed the expression of GABA ionotropic receptors in different MeCP2 disturbance scenarios of crescent complexity and maturation. Starting from immortalized N2A cells and mice primary cortex cultures in which MeCP2 expression was knocked down with specific shRNAs, we observed a straightforward relationship between MeCP2 expression and GABA ionotropic receptors. Biochemical analysis (Western Blot) of core synaptic proteins in female control and MeCP2+/- mice brain cortex at two developmental stages (both pre and post-symptomatic) recapitulated the altered expression of the GABA receptor subunit A1 (GABRA1) in Rett samples, and pointed towards the importance of the developmental status on such relationship. Finally, post mortem patients' brains analysis through RNAseq ratified the importance of time frames when studying MeCP2 and Rett syndrome unbalanced neurotransmission. Our results point towards GABRA1 subunit, and by extension, to GABA receptors, as an interesting potential therapeutic target for GABAergic neurotransmission modulation in Rett Syndrome, enhancing the importance of early neurotransmission intervention, as some molecular changes appear even before the symptomatic presentation of the disease.

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NOTES

O.36. TRAFFIC LIGHTS PEPTIDES: A TOOL TO PHOTOCONTROL CLATHRIN-MEDIATED ENDOCYTOSIS

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Clathrin-mediated endocytosis (CME) is crucial to all eukaryotic cells. It is implicated in a variety of cellular processes that range from nutrient uptake, signal transduction and regulation of the membrane components including surface proteins. The functioning of this transient machinery requires a complex network of proteins that cannot be untangled only by means of genetic modification and immunological depletion. Although pharmacological tools can aid studying the dynamics of biological responses by acute inhibition or stimulation of the upstream processes, the freely diffusing nature of these molecules poses limits on the control of their activity. In this sense, photopharmacology offers powerful tools to manipulate endogenous processes with high spatio-temporal resolution and in a non-invasive manner. Traffic Lights (TLs) peptides were designed to be cell-permeable, photoswitchable inhibitors of the main adaptor complex in the CME machinery. Based on the structure of β-arrestin C-terminal peptide (BAP-long), they bind to the β-appendage of AP2 (β-adaptin), which mediates the binding of clathrin to the membrane or to cargo receptors. When tested in mammalian cells, TL1 and TL2 were spontaneously internalised and proved capable of inhibiting CME in a light-regulated manner (Nevola et al. 2013; Martín-Quirós et al. 2015). Here we show that TL peptides can be used as a tool to further investigate the molecular mechanisms behind CME in an extremely versatile eukaryotic model system, i.e. yeast. *S. cerevisiae* cells were deprived of the cell wall and the resulting spheroplasts were allowed to internalise the peptides as confirmed by fluorescence microscopy imaging. Subsequently, mutants expressing fluorescently tagged Sla1 - a coat-associated endocytic protein - were used to observe kinetic delays in the dynamics of vesicle formation, confirming the possibility of photoregulating CME events by means of TL2.

NOTES

O.37. CHRONIC OTOTOXICITY OF THE INNER EAR CAUSES A LOSS OF SYNAPSES AND FUNCTIONALITY IN MICE

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Animal studies of the inner ear generally examine ototoxic effects after acute or short-term exposure, and so a chronic ototoxic exposure model was developed in order to study the cellular and molecular event differences involved in this toxicity. Male 129S2/SvPasCrl mice were exposed to 30mM 3,3'-iminodipropionitrile (IDPN) in drinking water for 4 or 6 weeks; auditory and vestibular function were assessed via auditory brainstem responses (ABRs) and an established vestibular test battery. Control and treated animals were sacrificed after the exposure period, and cochlear and vestibular dissections were performed in order to study synapse loss with immunofluorescence analysis by confocal microscopy.

Auditory and vestibular dysfunction appeared progressively during the IDPN exposure. Confocal microscopy of the vestibular epithelia of the utricle noted the dismantlement of the calyceal junctions for type I hair cells (loss of caspr1) and a loss of synapses (decreased ribeye and/or GluA2 puncta) overall. In addition, there was a loss of synapses in the inner hair cells of the organ of Corti within the cochlea; loss occurred in a tonotopic manner, beginning from higher frequencies and progressing towards lower frequencies. The physical loss of outer hair cells was observed in the cochlea as well, occurring in a progressive tonotopic manner during intoxication. In conclusion, synaptic modifications are early events in the inner ear preceding hair cell loss during chronic ototoxicity in the mouse.

NOTES

O.38. DISSECTING THE VESTIBULAR CIRCUITRY OF MOTION SICKNES

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The vestibular system has classically been associated with balance and Motion Sickness (MS). It is widely accepted that MS develops with the occurrence of neural mismatches between the integrated input of sensory information under motion and the memory of previous experience. MS is characterized by an unpleasant feeling resulting from an alteration in autonomic functions, such as a reduction in ambulatory activity, food intake and body temperature. Accordingly, vestibular stimulations also induce these autonomic changes, recapitulating MS. However, a genetically-defined, specific vestibular nuclei (VN) circuitry involved in the regulation of autonomic functions has not been established. Here, we have used an optogenetic approach to dissect the vestibular connectome regulating physiological responses, modulating *in vivo* the activity of glutamatergic vestibular somas and select projections in freely-moving mice. Our results demonstrate that stimulation of glutamatergic vestibular neurons is sufficient to reduce motor activity, body temperature and intake of low-palatability food of regular chow. Interestingly, no food intake decrease was observed using a highly palatable diet, revealing a motivational component and ruling out physical impairment. Furthermore, using cell-type connectomics we identified glutamatergic vestibular projections to ventral posteromedial thalamic nucleus (VPM), ventromedial hypothalamic nucleus (VMH) and central amygdala (CeA). Moreover, we highlight the importance of the glutamatergic vestibulothalamic VN->VPM circuit in appetite suppression. Further analysis is undergoing to complete the glutamatergic vestibular connectomic definition and address the specific contribution of its projections. Overall, this data underscores a potential role of the glutamatergic vestibular connectome in the development of Motion Sickness.

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NOTES

SESSION 4A

NEURODEGENERATIVE DISEASES II

O.39. INVOLVEMENT OF SMALL RNAS IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE: FOCUS ON SCAG SPECIES

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Huntington's disease (HD) is a trinucleotide repeat expansion disease (TRED) caused by an expansion of CAG repeats in the coding region of huntingtin (htt) gene, leading to an expanded polyglutamine track in the htt protein. Classically, HD pathogenesis has been linked to abnormal function of the mutant protein through misfolding and aggregation. However, a detrimental role of the expanded CAG repeats at RNA level is gaining importance. It has been shown that CAG repeat small RNAs of approximately 21 nucleotides (sCAG) are produced in HD human brains and are neurotoxic in vitro. It has also been demonstrated that small RNA derived from the putamen of post-mortem HD patients (sRNA-HD) present a detrimental role that is partially reversed using a locked nucleic acid modified anti-sense oligonucleotide targeting the CAG repeat (LNA-CTG). Considering these results, we have analyzed the detrimental role of pure sCAG species in terms of behavioral and molecular outcomes. Moreover, it is addressed the possible contribution of an alteration in protein synthesis caused by sRNA-HD and more specifically, sCAG pure species. Our results indicate that sRNA present in the putamen of HD patients are playing a neurotoxic role that can be partially attributed to the sCAG produced by the processing of the expanded RNA. In addition, we propose a dysregulation of protein synthesis as a novel key event in HD pathogenesis. In conclusion, the present data opens a novel and promising door for new therapies for HD, not only focused in lowering mutant htt expression at a protein level, but also blocking the detrimental activity of expanded CAG repeat RNA.

NOTES

O.40. CEREBELLAR TRANSDUCTION OF ASTROCYTES AS GENE THERAPY STRATEGY FOR MEGALOENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS (MLC)

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Efficient cerebellar transduction is important for the treatment of diseases affecting motor function and ataxias among other neurological disorders, but it has not been sufficiently explored so far. With the aim to achieve global and robust gene expression in the cerebellum, we tested different delivery routes of administering viral vectors, like direct intraparenchymal injection, lumbar intrathecal administration or subarachnoid injection to the cerebrospinal fluid (CSF). For that purpose, we used an AAVrh10 vector driving the expression of GFP under the regulation of the GFAP promoter, specific for glial cells. We found that subarachnoid injection in the CSF at cerebellum was the most efficient route in transducing Bergmann's glia, severely affected in Megaloencephalic leukoencephalopathy with subcortical cysts (MLC), a neurological disorder characterized by macrocephaly, deterioration in motor functions, cerebellar ataxia and mental decline. It is a type of vacuolating myelinopathy and its diagnosis is through the identification of swollen brain with diffuse white-matter abnormalities and subcortical cysts, mainly in temporal regions. AAVrh10 coding for MLC1 under the control of the GFAP promoter was injected it in the subarachnoid space of the cerebellum of MLC KO and wild type animals at 2 months of age, before the onset of the disease, as a preventive approach, and at 5 or 15 months, once the disease has been already stablished, as a therapeutic strategy. Western-blot, quantitative RT-PCR and immunohistochemistry showed MLC1 expression in the cerebellum and extremely reduced myelin vacuolation, a hallmark of the disease, in treated 8-month old animals. In addition, GlialCAM, an Ig-like adhesion molecule, also implicated in MLC, restored its localization in Bergmann glia after AAV-mediated MLC expression. These results may have implications for gene therapy to treat MLC patients as well as for other diseases affecting the cerebellum.

NOTES

O.41. INCREASED LAMIN B1 LEVELS CONTRIBUTE TO ALTERATIONS IN NUCLEAR PERMEABILITY IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a dominant inherited neurodegenerative disorder caused by an unstable expansion of a CAG repeat within the exon 1 in the huntingtin (htt) gene, cursing, among others, with cognitive and motor alterations related to dysfunction in hippocampal and corticostriatal pathways. However, molecular mechanisms leading to selective neuronal dysfunction are not clear yet. Previous results of our group show an upregulation of lamin B1, a structural nuclear protein, in different brain regions of HD patients and mouse models, together with alterations in nuclear envelope morphology. All these evidences suggest that neurodegeneration could be associated with alterations in the nuclear lamina. Through Fluorescence-Activated Nuclear Suspension Imaging (FANSI), we aimed to identify the specific neuronal populations affected in each brain region. We found that lamin B1 protein is specifically increased in striatal medium-sized spiny neurons (MSN) and neurons from the CA1 hippocampal region. Together with increased lamin B1 levels, neurons displayed an alteration in nuclear morphology, suggesting that altered lamin B1 levels may lead to alterations in nuclear structure that could play a role in HD symptoms. A possible mechanism by which the increase in lamin B1 protein levels could be involved in HD pathology is by altering nuclear permeability. We used Fluorescence Recovery After Photobleaching (FRAP) to evaluate nuclear permeability of MSN. Our results indicate a slower entrance of 20KDa-dextran in R6/1 neuronal nuclei compared to WT, indicating a decrease in nuclear permeability in MSN from R6/1 mice. Altogether, our results show that the increase in lamin B1 protein levels may participate in the pathophysiology of HD by altering nuclear permeability and may contribute to the selective neuronal dysfunction described in HD pathology.

NOTES

O.42. DRP1-MITOCHONDRIAL FRAGMENTATION CONTRIBUTES TO DISRUPTING MITOCHONDRIA-ER CONTACT SITES IN HUNTINGTON'S DISEASE MOUSE STRIATUM

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Huntington's Disease (HD) is a neurodegenerative disorder characterized by the selective loss of striatal medium spiny neurons. Although the molecular mechanisms of this striatal vulnerability remain unclear, compelling evidence point out that mitochondrial dysfunction could play a major role. Mitochondria are essential organelles for neuronal survival since they are the source of intracellular ATP and regulate Ca2+ homeostasis. For ensuring the maintenance of their function, a balance between fusion and fission are strictly controlled. Interestingly, defects in mitochondria dynamics have been found in HD, where fission protein Drp1 activity is enhanced.

In the current work, we demonstrate in HD primary striatal neurons that excessive mitochondria fragmentation is accompanied by an unusual distribution of these organelles far from the endoplasmic reticulum (ER). Moreover, using a proximity ligation assay (PLA), we also observed a reduction in contact sites (MAMs) between both organelles. Interestingly, pharmacological inhibition of Drp1 by Mdivi-1 results in restoring both mitochondrial fragmentation and ER-mitochondria interactions in HD primary striatal cultures. On the other hand, it is known that MAMs enable calcium effluxes from the ER to the mitochondria via VDAC-IP3R3 complex, reinforced by Grp75 and Mfn2 proteins. In this study, specific changes of MAM-resident Ca2+ regulatory proteins were detected by Western Blot in the striatum of HD transgenic mice along the disease progression and in HD human brain. Taken together, these data support the hypothesis that mitochondrial dynamics disruption may contribute to a major susceptibility of the striatum to huntingtin toxicity by altering mitochondria and ER contacts.

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NOTES

O.43. ROLE OF NEUROMELANIN IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease clinically defined by a combination of motor features that include bradykinesia, rigidity, tremor and postural instability. These symptoms are attributable to a massive and selective dopaminergic neuronal loss in the *substantia nigra pars compacta* (SNpc), a specific brain region named for the presence of a dark-brown pigment, neuromelanin (NM), which can be seen macroscopically as a darkened area. NM progressively accumulates in catecholaminergic neurons until occupying most of the cytoplasm, since neurons apparently lack the mechanisms for eliminating this pigment. Distinct cell groups within ventral midbrain differ markedly from each other in the percentage of pigmented neurons they contain and this percentage directly correlates with estimated cell loss in PD. Furthermore, Lewy bodies, the pathological hallmark of PD together with nigral neurodegeneration, typically appear associated to NM granules. Likewise, neuroinflamatory changes in PD are particularly localized within NM-containing regions. In addition, NM accumulates progressively with age, the latter being the main risk factor for developing PD. In spite of such an intimate association between NM and PD, the physiological significance of NM and its potential contribution to PD pathogenesis has been largely neglected so far in experimental *in vivo* paradigms of the disease. This is mainly because, in contrast to humans, laboratory animal species commonly used in experimental research, such as rodents, lack NM. To address this major limitation, we generated a "humanized" rat model exhibiting age-dependent accumulation of human-like NM in SNpc DA neurons, up to levels comparable to those reached in elderly humans. Using this model, we assessed how progressive NM accumulation in SNpc may affect neuronal function and viability.

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NOTES

O.44. ANALYSIS OF EPIGENETIC ALTERATIONS IN SAMP8, A MODEL OF AGE RELATED COGNITIVE DECLINE

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A growing body of research shows that epigenetic mechanisms are critically involved in normal and pathological aging. The SAMP8 mouse model can be considered a useful tool to better understand the dynamics of the global epigenetic landscape during the aging process since its phenotype is not fully explained by genetic factors. Here we investigated dysfunctional age-related transcriptional profiles and epigenetic programming enzymes in the hippocampus of 2- and 9-month-old SAMP8 female mice using the SAMR1 strain as control. SAMP8 mice presented 1,062 genes dysregulated at 2-month-old, (early-onset genes) and 1,033 genes at 9-month-old (late-onset genes), with 92 genes concurrently dysregulated at both ages. SAMP8 mice showed a significant decrease in global 5-mC at 2 months while 5-hmC levels were increased in SAMP8 at 2 and 9 months of age compared to SAMR1. These changes were accompanied by changes in the expression of several enzymes that regulate DNA methylation and methylcytosine oxidation. Acetylated H3 and H4 histone levels were significantly diminished in SAMP8 at 2-month-old compared to SAMR1 and altered HDAC profiles were detected in both young and old SAMP8 mice. We analyzed 84 different mouse miRNAs known to be altered in neurological diseases or involved in neuronal development. Compared with SAMR1, SAMP8 mice showed 28 and 17 miRNAs were differentially expressed at 2 and 9 months of age, respectively, 6 of these miRNAs overlapped at both ages. We used several bioinformatic approaches to integrate our data in mRNA:miRNA regulatory networks and functional predictions for young and aged animals. In sum, our study reveals interplay between epigenetic mechanisms and gene networks that seems to be relevant for the progression towards a pathological aging and provides several potential markers and therapeutic candidates.

NOTES

O.45. EFECTOS DE APOE SOBRE LA COGNICIÓN Y SU INTERACCIÓN CON FACTORES DEMOGRÁFICOS Y BDNF

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La apolipoproteína E (ApoE) tiene un relevante papel en las múltiples trayectorias que presenta el declive de las funciones cognitivas asociado a la edad. No obstante, el impacto del gen está mediado por múltiples factores. El objetivo de nuestro estudio ha sido investigar el efecto de los diferentes alelos de ApoE sobre la cognición y su interacción con el sexo, edad y el gen BDNF en una muestra de 747 sujetos sanos mayores de 50 años. Se genotiparon polimorfismos de un solo nucleótido (SNPs) en los genes BDNF y ApoE y se realizó una evaluación neuropsicológica a los sujetos. Las diferentes pruebas cognitivas fueron agrupadas mediante análisis factorial en tres dominios cognitivos. Se estudió el correlato neurobiológico mediante resonancia magnética en una submuestra de 16 sujetos divididos en portadores del genotipo E2E3 (n=8) y e3e4 (n=8) pareados por sexo, edad y escolaridad.

Nuestros resultados mostraron mejor rendimiento en los portadores de ApoE2 en memoria verbal (B= 0,333; p=0.0032) y fluencias (B= 0,325; p=0.0021). Los portadores de ApoE4 presentaron mejor resolución visoespacial (B= 0,147; p=0.031). Cuando se estudió el efecto del sexo observamos que el efecto beneficioso de ApoE2 solo se daba en mujeres en memoria verbal (B= 0,739; p= $2{,}3\cdot10^{-5}$) y fluencias (B=0,565; p= $1{,}9\cdot10^{-4}$). La edad no mostró diferencias significativas. Finalmente se hallaron interacciones entre BDNF y ApoE2 en el dominio visoespacial (B= -0,698; p=0.005) y con ApoE4 en memoria verbal (B=0.285; p=0.043).Mediante DTI se observaron diferencias no corregidas entre E2E3 y E3E4 en fórnix, esplenio izquierdo del cuerpo calloso, tracto corticoespinal izquierdo, putamen y fascículo uncinado izquierdo y bulbo raquídeo, sugiriendo una mayor integridad de sustancia blanca en los portadores de E2.

ApoE E2 es un factor protector de memoria verbal y fluencias en una muestras de sujetos sanos mayores de 50 años. Además, el efecto protector de E2 sobre la cognición es especialmente beneficioso en mujeres.

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NOTES

POSTER SESSION

GLIAL CELLS AND INFLAMMATION

P.1. PARKINSONIAN NEUROTOXINS IMPAIR GLIAL CELL IMMUNE RESPONSE

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It is widely accepted that glial activation/neuroinflammation is involved in the development of neurodegenerative disorders. However, its role in the etiopathogenesis and the progression of each disease remains to be stablished. Microglial cells, the main representatives of the endogenous immune system of the CNS, have a capital role on the brain innate immune response. When alterations in brain homeostasis occur, surveillant microglial cells become quickly activated and develop morphological and functional changes aimed to recover brain homeostasis and function. Different patterns of glial activation are associated to specific changes in glial metabolism. An appropriate immune response is critical for normal brain function. It is suggested that an impaired immune response is responsible for neuroinflammation occurring in neurodegenerative diseases. In a previous study, we showed that the parkinsonian neurotoxins MPP+ and rotenone, inhibitors of the respiratory chain, induce alterations in the response of glial cell cultures to a pro-inflammatory stimulus. The aim of the present work was to study the effect of these agents on the development of an anti-inflammatory response in glial cells. Mouse primary glial cultures (mixed glial and microglial cultures) were treated with MPP+ or rotenone in the absence and the presence of the classical anti-inflammatory stimulus IL4. We evaluated cell viability, expression of anti-inflammatory markers, phagocytic activity, and glial cell metabolism.

MPP+ and rotenone induced morphological and functional changes in glial cells. They inhibited the metabolic activity in mixed glial cultures. In addition, they inhibited the anti-inflammatory response in IL4-stimulated glial cells. Consequently, glial cells exposed to MPP+ or rotenone display an altered functionality that could result in an impaired immune response. These results suggest that the exposure to agents that alter glial metabolism may impair brain immune response, which may contribute to neuronal damage in Parkinson's disease and other neurodegenerative disorders.

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P.2. EFFECTS OF THE OVEREXPRESSION OF IL-6 AND IL-10 ON THE CD200/CD200R SIGNALLING AFTER THE FACIAL NERVE AXOTOMY

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Facial nerve axotomy (FNA) is a well-known model of sterile neuroinflammation. Research developed in our laboratory showed that after FNA, animals overexpressing IL-6 (GFAP-IL6Tg) had an increase in motor neuron cell death, whereas animals overexpressing IL-10 (GFAP-IL10Tg) presented an increase in motor neuron survival and, in both cases this outcome was related to a broad microglial activation. Neuron-microglia communication is performed through signals such as CD200/CD200R, and therefore, this axis could play a key role after FNA. The aims of this study were to analyse the regulation of CD200/CD200R after FNA and to explore the effects of the overexpression of IL-6 and IL-10. With this purpose, adult GFAP-IL6Tg, GFAP-IL10Tg and WT mice were lesioned and sacrificed at 3, 7, 14, 21 and 28 dpi. Single and double immunohistochemical techniques were used to detect and quantify CD200 and CD200R. Our results showed that in the facial nucleus, CD200 is mainly expressed on neurons and CD200R on microglia and perivascular macrophages. In basal conditions, no differences were observed on CD200/CD200R levels between transgenic and WT animals. After FNA, CD200 and CD200R are upregulated reaching a peak at 7dpi suggesting an attempt to regulate microglial activation, followed by a decrease at 14, 21 and 28dpi in both CD200 and CD200R, during and after the neuronal death peak. Whereas IL-10 overexpression exerts no effects on CD200 and CD200R, IL-6 overexpression downregulates CD200R levels at later timepoints, which correlate with an increase in microglial activation. These results suggest that an anti-inflammatory environment does not have any effect on this signalling while a proinflammatory environment decreases CD200R levels after FNA indicating that this could be related to a higher neuronal death. In summary, induction of CD200R signalling in pro-inflammatory conditions could constitute an interesting target to control neuroinflammation.

P.3. EFFECT OF LOCAL CNS PRODUCTION OF EITHER IL-6 OR IL-10 IN MICROGLIA PROLIFERATION AFTER FACIAL NERVE AXOTOMY

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In the experimental model of facial nerve axotomy (FNA), a slow motor neuron death is triggered in facial motor neurons, concomitantly with microglia activation and cluster formation. Previous studies in our group reported that transgenic mice with an astrocyte-targeted production of interleukin-6 (GFAP-IL-6Tg) showed a decreased formation of microglial clusters and increased motor neuron death respect to wild-type (WT) mice, while mice with local CNS production of interleukin-10 (GFAP-IL-10Tg) showed an increased microglial cluster formation and decreased motor neuron death. Microglial clusters are known to be involved in phagocytosis, however, their participation in microglia proliferation is unclear. Thus, the aim of this work was to study microglia proliferation after FNA in WT, GFAP-IL-6Tg and GFAP-IL-10Tg mice, and the involvement of microglial clusters in this process. With this purpose, GFAP-IL-6Tg, GFAP-IL-10Tg and WT mice underwent FNA and samples from 3 to 28 days post injury (dpi) were studied using immunohistochemistry with antibodies directed against Pu.1, phosphoHistone-3 and Iba-1. Our results showed that microglia cell density increased until 14 dpi and decreased at later timepoints in all mouse lines. In GFAP-IL-6Tg, a significantly higher microglia cell density number was observed in basal conditions respect to WT mice, while in GFAP-IL-10Tg, increased microglial cell density was found at 14 dpi. In accordance with changes in microglial density, a high microglial proliferation was observed at 3 dpi, that decreased importantly at 7 and 14 dpi and was absent later in WT mice, as well as in transgenic mice. Additionally, results indicated that microglial clusters were formed at 14 dpi and decreased thereafter, although in GFAP-IL-6Tg spare clusters were observed before. In conclusion, our results suggest that local CNS overproduction of IL-6 and IL-10 do not produce important modifications in microglia proliferation, and microglial clusters formed after FNA are not involved in this process.

P.4. INTRACELLULAR CA^{2+} IN THE PEPTIDERGIC SECRETION OF UNSTIMULATED ASTROCYTES

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Astrocytes are specialized glial cells that can communicate within the CNS through the release of vesicular transmitters triggered by a rise in intracellular Ca^{2+} levels. Differently to neurons, which display a very low basal secretion and a higher stimulus-evoked release of neuropeptides, stimulated secretion of astrocyte peptides is moderated and exhibits a substantially high basal release. In this study, we evaluated the role of intracellular Ca^{2+} in the secretion of peptidergic transmitters of unstimulated astrocytes in vitro. In astrocyte cultures, immunocytochemical analyses revealed that astrocyte-contained secretory proteins such as carboxypeptidase E, secretogranin III, lipocalin 2 and cystatin C were distributed in vesicle subpopulations. To monitor the retention and secretion kinetics of newly generated peptidergic transmitters, a cycloheximide (CHX) chase assay was performed in astrocytic cultures. Untreated cells displayed an increase in extracellular and invariable intracellular levels of peptidergic transmitters over time. After the blockage of protein synthesis by CHX, intracellular levels of secretory peptides decreased gradually whilst its extracellular levels accumulated progressively, suggesting that de novo synthesized astrocytic secretory peptides undertake a low retention and are rapidly released in unstimulated cells. Furthermore, similar release assays were carried out using the cell-permeable Ca^{2+}-chelator BAPTA-AM. When protein synthesis was blocked, the release of peptidergic transmitters in the presence of BAPTA-AM was dramatically decreased, while their intracellular levels were elevated, suggesting that in the absence of intracellular Ca^{2+} secretory peptides are highly accumulated. These findings point towards intracellular Ca^{2+} playing a role in the peptidergic secretion of unstimulated astrocytes. Currently, we are investigating whether over-time sustained high levels of cytosolic Ca^{2+} or spontaneous Ca^{2+} oscillations driven by a culture-intrinsic stimulation could explain the observed release patterns.

P.5. TRANSGENIC IL-6 AND IL-10 MODIFY THE EXPRESSION OF MICROGLIAL PHAGOCYTIC RECEPTORS INVOLVED IN MYELIN RECOGNITION DURING AGING

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During aging, microglial cells undergo specific changes related to a pro-inflammatory microenvironment. One of the functions that are altered is their phagocytic capacity. This microglial phagocytosis in the aged brain has been characterized by accumulations of lysosomes with an inefficient degradation of their content. In addition to microglial alterations, a recent study has reported accumulation of myelin fragments in the white matter of aged mice. In this context, the aim of this study is to analyse the expression of microglial receptors related to myelin recognition during physiological aging and under the overexpression of the pro-inflammatory interleukin-6 (IL-6) and the anti-inflammatory interleukin-10 (IL-10). For this purpose, adult (4-6 months old) and aged (18-21 months old) mice from two different transgenic lines with astrocyte-targeted production of either IL-6 (GFAP-IL6Tg) or IL-10 (GFAP-IL10Tg), and their corresponding wild-type littermates (WT), were used. Compared with aged WT animals, microglial cells from both aged transgenic lines showed a more activated microglial phenotype and higher cell density. These changes have been found in both grey and white matter areas. Expression of CD68, a marker of macrophages/microglia lysosomes, was high in both aged WT and aged transgenic mice. However, while in aged WT, CD68 was restricted to the microglial soma, in both aged transgenic animals it was redistributed along microglial cell branches indicating an alteration in the distribution of lysosomes. Interestingly, our results also demonstrated a de novo expression of Galectin-3 and an increase of TREM-2 in white matter areas of aged WT animals. Expression of these two receptors was higher in aged GFAP-IL6Tg, but was not modified in aged GFAP-IL10Tg. Our results correlate with the myelin sheets alterations previously reported in aged mice, and show an impact of the microenvironment in the microglial phagocytic capacity during aging, with a different expression of receptors involved in myelin recognition.

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P.6. CREB INHIBITION SHAPES CALCIUM RESPONSES IN ASTROCYTES *IN SITU*

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Calcium responses are considered the base of astrocytic excitability allowing them to communicate with neurons. Previously, we have shown long-term adaptative plasticity like phenomena in cultured astrocytes mediated by the transcription factor CREB. Here we aim to analyze the effect of CREB astrocytes in situ. We generated serotype 9 adenoassociated viral vector (AAV9) codifying for a dominant negative CREB construct (A-CREB) and the red fluorescent TdTomato protein. Null AVV9 codifying only for TdTomato were also produced. A-CREB and Null AAVs were injected together with the fluorescent calcium indicator AAV2/5-gfaABC1D-GCamp6f in the hippocampus of C57/Bl6 mice. A control group of mice were infected only with the calcium indicator viral vector. Three weeks later, brains were dissected out and slices prepared to analyze calcium signals by confocal microscopy. Spontaneous activity was similar between the three groups of animals (A-CREB, Null and GCamp6f-only). No differences were seen in frequency (the number of events per minute) or amplitude (the height of the peak) of calcium responses in the soma or primary branches of astrocytes. However, differences were seen in the half width parameter (duration of the event) of A-CREB peaks compared to Null and GCamp6f-only mice, with a clear tendency in the somatic events and significantly different in the branches. Likewise, when the α1-adrenergic receptor agonist phenylephrine was added, significant differences in A-CREB animals were seen in amplitude of the response (area under the curve), especially in the soma. The amplitude of the peak of phenylephrine-induced calcium responses was similar between the three mice groups both in somatic and branches regions. By contrast, when the purinergic receptor agonist ATP was added, A-CREB mice had no differences in the calcium responses compared to control mice. All together our results show that inhibition of endogenous CREB in hippocampal astrocytes modulates particular calcium responses in situ.

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NEURODEVELOPMENT AND STEM CELLS

P.7. GENERATION OF NEUROTROPHIC FACTOR-RELEASING STEM CELLS AS A CELL-BASED APPROACH TO TREAT NEURODEGENERATIVE DISEASES

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Huntington's disease (HD) is a neurodegenerative disease caused by the toxic mutant huntingtin (mhtt) protein. HD primarily affects the medium spiny neurons (MSNs) of the striatum. Brain-derived neurotrophic factor (BDNF) can protect those neurons but its expression is compromised in the presence of mhtt. Here, we propose a cell-based therapy to repair or replace the damaged MSNs by the co-transplantation of striatal neuron precursors differentiated from pluripotent stem cells, and mesenchymal stem cells (MSCs) engineered to express BDNF. This approach is based on the encouraging outcomes obtained from in vivo applications of BDNF and the known immunomodulatory capability of human bone marrow MSCs (hBM-MSCs). Here we develop the tools to genetically engineer hBM-MSCs to overexpress BDNF. Two strategies have been designed: a constitutive expression system and an inducible expression system that requires doxycycline to activate expression. hBM-MSCs are transduced using lentiviral particles and the production and release of BDNF is quantified by ELISA and western blot. We anticipate that the transplantation of neuronal precursors in combination with BDNF-expressing hBM-MSCs has the potential to restore the damaged MSNs in HD. Such an approach could also be used to treat other neurodegenerative diseases that also display a devastating loss of neurons.

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P.8. STRIATAL AND THALAMOCORTICAL AXONS DEFECTS IN RHOEGT/GT EMBRYONIC BRAINS

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RhoE is a RhoGTPase involved in axon growth and neuron migration. RhoE knock-out mice display neurodevelopmental impairments, such as lack of the common peroneal nerve and reduction of calbindin-expressing neurons in the olfactory bulb. The aim of this study is to analyse the in vivo role of RhoE in brain connectivity development. During development, immunofluorescence and axon tracing analysis revealed that RhoEgt/gt mice showed severe axonal projection defects: TCAs were unable to cross the diencephalon-telencephalon boundary (DTB) and striatonigral axons (SNAs) were misguided ventrally. Surprisingly, the corridor was not severely affected. Deeper analysis of subpallium revealed that ventral striatum Islet1/RhoE+ cells were mispositioned ventrally, together with Globus Pallidus and striatal axons. We confirmed a similar but less severe phenotype in conditional nervous system (NestinCre-RhoElx/gt), striatal-LGE-MGE (Dlx5/6Cre-RhoElx/gt) and MGE-specific (Nkx2.1Cre -RhoElx/gt) knock-out brains. We propose that RhoE is important for the proper migration of Islet1+ neurons in the striatum and the GP formation. In RhoE absence, these neurons are mislocalized disturbing SNA projections, which in turn may affect secondarily the guidance of TCAs through the ventral subpallium. In summary, our results suggest I) an important role of RhoE in the correct development of subpallium; II) that proper positioning of GP cells is needed for a correct SNAs guidance and III) a scaffolding role of SNAs to help TCAs crossing the DTB.

P.9. AGE-DEPENDENT SECRETION HETEROGENEITY OF DENSE-CORE VESICLE SUBPOPULATIONS IN HIPPOCAMPAL NEURONS

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Dense core vesicles (DCVs) release a variety of transmitters in a calcium-regulated fashion to control development, plasticity and physiology of neural circuits. In response to a physiological signal the secretion of DCV-stored neuropeptides, peptidic hormones and growth factors is triggered by regulated exocytosis. Previous studies supported the idea of differential packaging and routing of DCV cargo. However, the secretory mechanisms and protein profiles of DCVs are poorly known, especially during brain development. Here, we studied distribution and secretory dynamics of key DCV components in the developing and adult cerebral cortex. Proprotein convertases PC1/3 and PC2, the carboxypeptidase CPE and the granin SgIII were analyzed in cultured and in situ cortical cells by immunofluorescence and western blotting procedures. In adult and postnatal tissues, CPE and SgIII were widely detected in neurons, astrocytes and progenitor cells, whereas PC1/3 and PC2 were exclusively localized in neuronal populations. Intracellularly, a heterogeneous distribution for DCV proteins was observed in neurons and astrocytes. A robust immunolabelling through the axonal domain was found for SgIII and PC1/3, but only CPE was associated with dendritic shafts. Triggering evoked release by different secretagogues showed similar responses for all DCV proteins studied in adult tissues but not at early postnatal stages. An expected release profiles were found for SgIII and CPE in postnatal brain slices and cortical cultures, whereas PC1/3 showed a transient differential secretion. Taken together, these results suggest that a heterogeneous DCV composition and secretion dynamics is required to drive development and function of the cerebral cortex.

NEURONAL CIRCUITS AND PLASTICITY

P.10. INCREASE IN HIPPOCAMPAL POSTSYNAPTIC DREBRIN PROTEIN EXPRESSION IN RATS WITH INTRACRANIAL SELF-STIMULATION TREATMENT CORRELATES WITH SPATIAL MEMORY PARAMETERS AND CORTICAL NEURAL ACTIVATION

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Electrical intracranial self-stimulation in the lateral hypothalamus (LH-ICSS) is a widely used technique to study the effects of rewarding stimuli and motivational behaviour, as it stimulates the reward circuit. LH-ICSS has also been shown to facilitate the acquisition and retention of spatial tasks dependent on the hippocampal system, in rats. The possible underlying mechanisms that have been proposed to explain the facilitative effects of LH-ICSS on memory include an increased structural plasticity and the expression of plasticity-related genes in the hippocampus and other memory-related structures. In the present study, we aimed to determine the effects of LH-ICSS on the expression of postsynaptic plasticity related protein drebrin (DBN), in relation to memory performance and neural activation. LH-ICSS took place immediately after each of the five learning sessions of a spatial Morris Water Maze (MWM) task. A retention test was performed 72h after the last LH-ICSS treatment. Animals were euthanized 90min after the MWM retention test, in order to analyse DBN in various hippocampal regions and c-Fos expression in several brain areas by means of immunohistochemistry. The results indicate that LH-ICSS increases the expression of the postsynaptic protein DBN in the suprapyramidal region (SP) of the dentate gyrus (DG) and in the stratum lacunosum moleculare (SLM) of CA1. In addition, DBN expression in the SP region of the DG seems to be linked to the behavioural outcome, as shown by correlational analyses. Additionally, c-Fos expression in the granular retrosplenial cortex (RSG) and prelimbic cortex (PL) was shown to be positively correlated to the strengthened postsynaptic plasticity in the hippocampus. In conclusion, LH-ICSS has been shown to promote postsynaptic plasticity in the rat's hippocampus, which correlates with increased neural activation in the RSG and PL, as well as with the animal's ability to learn and remember a spatial task in the MWM.

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P.11. SYNAPSE-TO-NUCLEUS SIGNALING MEDIATED BY THE CREB-REGULATED TRANSCRIPTION COACTIVATOR-1 (CRTC1) REGULATES NMDA-DEPENDENT SYNAPTIC PLASTICITY

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The capacity of neuronal activity to modify the strength or efficacy of synaptic transmission at synapses is known as synaptic plasticity. Activation and synaptic recruitment of glutamate N-methyl-D-aspartate receptors (NMDARs; GluN) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs; GluA) play key roles on hippocampus-dependent synaptic plasticity and memory. Synapse-to-nucleus signaling plays also a central function in long-term synaptic plasticity and memory by linking activation of synaptic NMDA receptors to gene transcription in the nucleus. How synapse-to-nucleus signaling mediates potentiation of GluNs back at stimulated synapses is still unclear. In this study, we used biochemical and cellular techniques and gain and loss of function gene expression approaches to elucidate the role of the synapse-to-nucleus factor CREB-regulated transcription coactivator-1 (CRTC1) in synaptic plasticity in the hippocampus. Adeno-associated viral-mediated CRTC1 overexpression or silencing in the adult mouse hippocampus affects differentially long-term potentiation and PKC/PKA-mediated phosphorylation of GluN1. Indeed, we show that CRTC1 plays essential roles on functional synaptic plasticity favorable to enhance long-term memory by modulating the phosphorylation of excitatory glutamate receptors. By contrast, CRTC1 does not affect levels of total and phosphorylated GluA1 and GluA2 subunits. These results suggest that the synapse-to-nucleus factor CRTC1 modulates functional synaptic plasticity by regulating PKC-dependent GluNs localization at excitatory synapses.

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P.12. ROLE OF RTP801 IN NEURONAL PLASTICITY AND MOTOR LEARNING

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RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death. RTP801 protein levels are elevated in cellular and animal modelsof Parkinson's disease (PD) andHuntington's disease (HD) and also in human postmortem brain samples from PD and HD patients. RTP801 is pro-apoptotic by sequentially inhibiting mTOR and Akt via the tuberous sclerosis complex (TSC1/2). RTP801 also has a regulatory role in cortical development, neuronal differentiation and peripheral nervous system myelination. In our preliminary results we observed that RTP801 is enriched at the synapses of both murine models and human postmortem brains. Here, we investigated whether RTP801 has a role in neuronal plasticity by using both cellular and animal models. We first characterized the RTP801 knock out (KO) mice versus wild type (WT) animals at 2 months of age at a behavioral, histological, electrophysiological and biochemical level. Although RTP801 KO animals showed no significant differences in locomotor activity they performed better in tasks involving motor learning. In line with this, RTP801 KO mice showed differences in spine density and morphology in specific brain areas and also in synaptic protein content. To investigate in further detail RTP801's role in neuronal plasticity, we also performed electrophysiological analyses in primary cortical cultures from RTP801 KO and WT mice. Altogether, these results suggest that RTP801 has an important role in modulating neuronal plasticity and motor learning.

P.13. TRANSCRIPTIONAL MECHANISMS OF CREB-REGULATED TRANSCRIPTIONAL COACTIVATOR-2 (CRTC2) IN THE BRAIN

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The transcription factor cAMP-response element binding protein (CREB) mediates neuronal excitability, synaptic plasticity and long-term memory in the hippocampus. Differential CREB-dependent transcription depends on its interaction with different coactivators, such as the histone acetyl-transferase (HAT) CREB binding protein (CBP) and the CREB-regulated transcription coactivators (CRTCs). CRTC family comprises three members (CRTC1, CRTC2 and CRTC3) that are differently expressed in mammalian tissues. Whereas CRTC1 mediates synaptic plasticity and memory, the role of CRTC2 and its regulatory mechanisms in the brain are still largely unknown. The aim of this study was to elucidate the mechanisms of CRTC2 regulation, and its role in CREB-dependent gene transcription in the adult brain. Histochemical analyses revealed that CRTC2 is mainly expressed both in neurons and astrocytes but not in microglia in the adult mouse brain. In cultured neurons, CRTC1 and CRTC2 isoforms are differently expressed during differentiation suggesting they could play distinct roles during neural development. Moreover, biochemical analyses revealed CRTC1 dephosphorylation/activation is blocked by inhibiting PP2B/calcineurin, whereas CRTC2 is blocked by inhibiting PP1. Surprisingly, in astrocytes, CRTC1 dephosphorylation is regulated by PP2B, whereas CRTC2 dephosphorylation is modulated neither by PP1 nor PP2B. To study the biological function of CRTC2 in the adult brain, we generated a novel neuronal-specific Crtc2 conditional knockout (cKO) mice. Crtc2 cKO mice are viable and show a significant reduction of CRTC2 levels in different brain regions at postnatal day 21. Crtc2 ablation does not seem to correlate with a CRTC1 or CRTC3 compensation although more biochemical analysis are required to determine the consequences of CRTC2 loss.

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P.14. CHANGES ON SYNAPTIC PLASTICITY-RELATED miRNAs AS A RESULT OF INTRACRANIAL SELF-STIMULATION

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Deep brain stimulation (DBS) is being evaluated as a treatment for Alzheimer's disease (AD). Intracranial self-stimulation (ICSS) is a rewarding type of DBS that has shown facilitating effects on learning and memory, as well as modulation in the expression of plasticity-related proteins. However, the molecular bases of ICSS are not clearly understood. MicroRNAs (miRNAs) have emerged as key regulators of gene expression, so their alteration could be part of ICSS mechanism. In this context, we analysed the suitability of reported endogenous control candidates and the miRNA altered expression in the hippocampus post-ICSS. Rats implanted with an electrode at the medial forebrain bundle were treated with three 45-minute ICSS sessions, on 3 consecutive days. The levels of miR-132-3p, miR-134-5p, miR-181c-5p and miR-146a-5p in CA1 and DG hippocampal subfields were quantified by qRT-PCR, at 30, 90 and 180 minutes after the last ICSS or Sham session (N=5-6 rats/group). MiR-16-5p resulted in a suitable endogenous control, according to both NormFinder and GeNorm algorithms. After normalization, we found a decreasing tendency for miR-146a ($p=0.08$), which has shown to be upregulated in the hippocampus of AD patients, in the DG region at 90 min post-ICSS. MiRNA expression profile at DG was further analysed at 90 min (N=12 rats/group), using a Taqman miRNA Panel. After a global normalization, the altered candidates ($FC\pm1.3$, $p<0.05$), e.g. miR-495-3p and miR-485-3p, were functionally analysed using DIANA-miRPath v3.0. Among their predicted targets there are important players in synaptic plasticity, such as BDNF and Sirt-1, as well as molecules involved in AD pathology, such as APP and BACE. Interestingly, western blot analyses also showed increased Sirt-1, 90 min post-ICSS ($p=0.01$).

These results suggest that ICSS causes an alteration in miRNA profile, affecting key players in plasticity that could have a role in the memory improvements shown after ICSS.

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P.15. HISTONE ACETYLATION DEREGULATION AS A MECHANISM OF COGNITIVE IMPAIRMENT IN DOWN SYNDROME

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Down Syndrome (DS) is the most common genetic intellectual disability arising from the trisomy of chromosome 21 (HSA21). Interestingly, several triplicated HSA21 genes are involved in epigenetic mechanisms, probably contributing to the gene expression deregulation and to the cognitive phenotype of DS. Indeed, epigenetic modifications are associated with cognitive functions and their deregulation is involved in many intellectual disabilities (IDs). Among them, histone acetylation is most robustly associated with cognitive potentiation. As a matter of fact, the use of histone deacetylase (HDAC) inhibitors enhances memory by promoting histone acetylation in several mouse models of IDs and neurodegeneration. We propose that a deregulation of histone acetylation in the DS brain could lead to reduced expression of cognition-related genes. If this is the case, histone deacetylases inhibitors (HDACis) could promote enhanced expression of those genes in brain regions involved in learning and memory thus minimizing some cognitive deficits in DS. To address the question of how the epigenome is affected in the DS brain, we present a cell-specific approach to avoid the "epigenetic noise" caused by the cell type heterogeneity of the whole tissue. Interestingly, here we show that the global acetylation levels of H3 are reduced in the hippocampus of the DS mouse model Ts65Dn. Additionally, we found that chronic treatment with the HDAC inhibitor SAHA promotes the degradation of HDAC2 in different brain regions and improves Ts65Dn memory performance. Altogether, our data support the idea that epigenetic imbalance, and more specifically histone acetylation deregulation, is involved in the DS cognitive phenotypes.

P.16. *IN VIVO* EVALUATION OF MICROVESSEL DENSITY BY MRI IN THE MOUSE BRAIN

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Cerebrovascular diseases are accompanied by alterations at the vascular level, which can lead to significant changes in vascular density over time. One of our final goals is the study of the chronic effects in the vasculature in aging mice. Microvessel density (MVD) has been described as a useful tool for the study of vascular changes associated with age, as well as different pathologies affecting the brain, such as tumors, stroke, or dementias of vascular origin, among others. The aim of this study is to ascertain if MVD is useful for the determination of vascular changes in a longitudinal manner. MVD can be quantitatively measured by calculation of the Q index. C57BL6 adult male mice were used for the different imaging modalities. Some animals underwent either transient or permanent occlusion of the middle cerebral artery. Magnetic resonance imaging (MRI) was used to collect T2w and T2w* maps using a 7T MRI magnet as required for MVD protocol. We successfully performed the MVD acquisition in vivo and longitudinally at four different time points over the course of two months. On the other hand, 3-dimensional imaging of solvent cleared organ (3DISCO) allows tissue clarification for the 3D image acquisition by microscopy at high-resolution. Confocal microscopy was used to obtain 3D images from 4mm-thick coronal brain slices, previously clarified by 3DISCO, which were analyzed by ROIs for comparison with MVD quantification. Images were analysed by MATLAB algorithms and ITK_SNAP.
Preliminary results indicate that MVD may be a useful method to quantify the evolution of vascular changes in the brain caused by the different pathologies, and could be a biomarker for the different stages of such pathologies, as well as for possible effects of therapeutic interventions.

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NEUROTRANSMITTER RECEPTORS AND NEUROPHARMACOLOGY

P.17. A NOVEL NMDA RECEPTOR ANTAGONIST, IMPROVES COGNITIVE PERFORMANCE THROUGH ACTIVATION OF BDNF PATHWAY IN SAMP8 MICE MODEL

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Alzheimer's disease (AD) is the main cause of dementia, a group of brain disorders with behavioral abnormalities and memory loss. Currently, there is no effective therapy able to cure or reduce the AD progression. Memantine, a non-competitive NMDA receptor antagonist improved in cognition and molecular alterations after preclinical treatments. Nevertheless, such neuroprotective effects of memantine in preclinical studies do not translate into clinical results. For this reason, our research group has synthesized a new NMDA channel blocker, RL-208 bearing an amine polycyclic scaffold. The present work evaluated the in vivo efficacy of the RL-208 in cognition and cellular pathways in SAMR1 and SAMP8. Males, 5 months-old, were divided into four groups: two control groups, SAMR1 and SAMP8, and two mice treated groups with both strains. RL-208 administered in drinking water, 5 mg/Kg per day during 4 weeks. Behavioral tests (Three Chamber Test (TCT), Novel Object Recognition Test (NORT) and Object Location Test (OLT)) were applied and molecular analysis was performed through Western blot, RT-PCR and Fluorimetric Assay in hippocampus tissue. After the treatment, behavioral changes in both treated-mice groups were found. Furthermore, better cognitive performance was found in SAMP8 treated with RL-208, whereas SAMR1 maintained cognitive functions. Consistent with behavioral results, RL-208 treated-mice groups significantly increased protein levels of antioxidant enzymes such as SOD1 and GPX1 as well as a reduction of hydrogen peroxide. Treated groups also showed increased levels of mBDNF and a prevention of TrkB-FL cleavage leading to synaptic plasticity. Besides, we found increased protein levels of Synaptophysin and PSD95. Remarkably, RL-208 decreased gene expression of Adam10 promoting the non-amyloidogenic pathway. In sum, these results demonstrate the neuroprotectant role of RL-208 through specific biological pathways related to aging and neurodegenerative diseases, having a potential therapeutic effect in brain disorders.

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P.18. BENEFICIAL EFFECTS OF 11B-HSD1 INHIBITION ON COGNITIVE PERFORMANCE IN METABOLIC STRESSED SAMP8 FEMALE

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Aging and obesity are a global epidemic that significantly affect population health. Aging is the greatest risk factor for a majority of chronic diseases, such as Alzheimer's disease (AD). In addition, obesity plays an important role in mild cognitive impairment and dementia. Mounting evidence suggests elevated glucocorticoid (GC) levels cause obesity, metabolic stress, diabetes and memory impairment, as well as affect a wide range of processes in the brain, altering neurotransmission, metabolism, cell division and death. It has been described that prolonged exposure to GCs excess exert deleterious effects on hippocampal electrophysiology, structure and function, being part of altered intercellular communication described as one of the nine hallmarks of aging. The hippocampus is a crucial area for memory formation and consolidation. Moreover, GCs are important regulators of lipid metabolism, promoting lipolysis with acute treatment, but on the contrary, lipogenesis with chronic exposure. 11β-HSD1 regulates the conversion of GCs from inactive to active forms, thus amplifying their action, widely expressed in adult CNS. In our previous work, we determined that inhibition of 11β-HSD1 enzyme promotes autophagy and correlates with cognitive improvement in SAMP8 model.

Here, we divided SAMP8 females into Control, 11β-HSD1i, HF and HF+11β-HSD1i groups. During 4 months, HF and HF+11β-HSD1i mice were fed with high-fat diet and the last month, an 11β-HSD1 inhibitor was administered at 21mg/kg. We determined cognitive improvement in both 11β-HSD1i treated mice groups. In consonance, it is observed a significant decrease in oxidative damage by enhancing antioxidant defence. Moreover pro-inflammatory markers were significantly reduced, as well as hyperphosphorylated tau levels in treated mice.

Therefore, its inhibition might be a positive influence both on cognition as well as metabolic syndrome. This study aims to evaluate inhibition of 11β-HSD1 mitigates oxidative stress (OS) and neuroinflammation induced by HF diet reducing hallmarks of cognitive aging in female SAMP8.

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P.19. NEUROPROTECTIVE EFFECT OF NOVEL IMIDAZOLINE I_2 RECEPTOR LIGANDS THROUGH MOLECULAR CHANGES IN MAPK SIGNALING AND SUPPRESSION OF THE APOPTOTIC PATHWAY

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Aims: I_2-Imidazoline receptors (I_2-IRs) are widely distributed in the central nervous system. I2IRs overexpression in the Alzheimer's disease (AD) patients' brain has been reported, indicating their implication in cognitive decline. This evidence suggests that potential high-affinity selective I2-IR ligands could contribute to the delay of the neurodegeneration. We have previously reported a neuroprotective and analgesic effects of new selective I2-IR ligands (MCR5 and MCR9). The present work aims to provide further molecular pathways involved in the neuroprotective effects of I2-IR ligands in In vitro and In vivo. Methods: Cerebellar granule cell cultures were used to study the MCR5/9 effect (25-500nM) cell viability (MTT) against cellular damage induced by serum potassium deprivation and 200µM hydrogen peroxide (H_2O_2) treatment. For in vivo studies, female 12months-old SAMP8 mice were divided into three groups: SAMP8 Control (SP8-Ct, n = 10), SAMP8 treated with 5 mg/Kg/day MCR5 (n = 8) or MCR9 (n = 8), administrated in drinking water for four weeks. The molecular analysis was performed by Western blot, RT-PCR and Fluorimetric AssayResults: The I_2-IR ligands mediated protection against cellular damage increasing cell viability. Remarkably, in vivo analysis showed that both compounds reduced caspase-3 levels as well as the enzyme's activity measured by α-spectrin breakdown fragments. Moreover, it was observed significant increased p-Akt/Akt ratio in MCR5 and decreased Erk ½ activation with increased levels of synaptic markers such as Synaptophysin and PSD95. Conclusions: These results demonstrate the neuroprotectant role for these new I2-IR ligands through specific molecular pathways, being promising therapeutic agents for age-related neurodegenerative disorders.

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P.20. NEW DRUG COMBINATION TO REDUCE MUSCLE ATROPHY

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Motor denervation is an important regulator of skeletal muscle mass and function. Denervation results in muscle atrophy that account for a decrease in the size of myofibers. Patients with traumatic nerve injury and motor neuron disease suffer from highly morbid denervation-associated muscle wasting for which there is no effective therapy. Several factors have been involved in the anabolism/catabolism balance in muscle atrophy, but effective therapeutic options are missing. We explore herein, the effects of treatment with a novel combination of two repurposed drugs in different models of muscle atrophy. Preliminary results show that this combination, but not its single components, reduces muscle weight loss in different long-term and short-term denervation-induced murine models. The mechanisms involved might be related to sirtuin 1 activation which is a known target of the combination. These findings offer therapeutic strategies to combat muscle atrophy.

P.21. PBF509, AN ADENOSINE A2A RECEPTOR ANTAGONIST WITH EFFICACY IN RODENT MODELS OF MOVEMENT DISORDERS

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Adenosine A2A receptor (A2AR) antagonists have emerged as complementary non-dopaminergic drugs to alleviate Parkinson's disease (PD) symptomatology. Here, we characterize a novel non-xhantine non-furan A2AR antagonist, PBF509, as a potential pro-dopaminergic drug for PD management. First, PBF509 was shown to be a high-affinity ligand at the human A2AR, since it antagonized A2AR agonist-mediated cAMP accumulation and impedance responses with KB values of 72.8 ± 17.4 nM and 8.2 ± 4.2 nM, respectively. Notably, these results validated our new A2AR-based label-free assay as a robust and sensitive approach to characterize A2AR ligands. Next, we evaluated the efficacy of PBF509 reversing motor impairments in several rat models of movement disorders, including catalepsy, tremor and hemiparkinsonism. Thus, PBF509 (orally) antagonized haloperidol-mediated catalepsy, reduced pilocarpine-induced tremulous jaw movements and potentiated the number of contralateral rotations induced by L-3,4-dihydroxyphenylalanine (L-DOPA) in unilaterally 6-OHDA-lesioned rats. Moreover, PBF509 (3 mg/kg) inhibited L-DOPA-induced dyskinesia (LID), showing not only its efficacy on reversing parkinsonian motor impairments but also acting as antidyskinetic agent. Overall, here we describe a new orally selective A2AR antagonist with potential utility for PD treatment, and for some of the side effects associated to the current pharmacotherapy (i.e. dyskinesia).

Keywords: PBF509, Parkinson's disease, adenosine A2A receptor, catalepsy, label-free, tremor, hemiparkinsonism.

P.22. ADENOSINE A_1-DOPAMINE D_1 RECEPTOR HETEROMERS CONTROL THE EXCITABILITY OF THE SPINAL MOTONEURON

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While the role of the ascending dopaminergic system in brain function and dysfunction has been a subject of extensive research, the role of the descending dopaminergic system in spinal cord function and dysfunction is just beginning to be understood. Adenosine plays a key role in the inhibitory control of the ascending dopaminergic system, largely dependent on functional complexes of specific subtypes of adenosine and dopamine receptors. Combining biophysical techniques such as bioluminiscent resonance energy transfer and bimolecular fluorescense, a selective destabilizing peptide strategy with a proximity ligation assay and patch-clamp electrophysiology in slices from male mouse lumbar spinal cord, the present study demonstrates the existence of adenosine A_1-dopamine D_1 receptor heteromers in the spinal motoneuron by which adenosine tonically inhibits D_1 receptor-mediated signaling. A_1-D_1 receptor heteromers play a significant control of the motoneuron excitability, represent main targets for the excitatory effects of caffeine in the spinal cord and can constitute new targets for the pharmacological therapy after spinal cord injury, motor aging-associated disorders and restless legs síndrome.

P.23. FUNCTIONAL DIFFERENCES BETWEEN HETEROMERS FORMED BY α_{1A} ADRENOCEPTORS AND DOPAMINE $D_{4.4}$ OR $D_{4.7}$ RECEPTOR VARIANTS COULD BE INVOLVED IN ADHD

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Attention deficit hyperactivity disorder (ADHD) is a prevalent neuropsychiatric disorder in children characterized by symptoms of inattention, hyperactivity and impulsivity. It has been demonstrated that the dopaminergic system is involved in ADHD, specially the dopamine receptor D_4 (D_4R) variant $D_{4.7}R$. The adrenergic system has also been linked to ADHD due its implication in visual attention, learning and memory. Many studies reveal that noradrenaline and dopamine have complementary effects in the reinforcement of prefrontal cortex (PFC) and striatum connections, suggesting that these coordinated effects could be mediated by interactions between their respective receptors. In particular, α_{1a} adrenoceptors ($\alpha_{1a}R$) and different variants of D_4R are expressed in PFC and striatum. We analysed whether $D_{4.4}R$ and $D_{4.7}R$ heteromerize with α_{1a} adrenoceptor, and the functional differences between D_4R variants. We have demonstrated $D_{4.4}R$-$\alpha_{1a}R$ and $D_{4.7}R$-$\alpha_{1a}R$ heteromerization in transfected cells by using Biolumiscence Resonance Energy Transfer (BRET) and in in rat striatum and cortex by in situ Proximity Ligation Assay (PLA). We also characterized the transmembrane domains (TM) implicated in $D_{4.4}R$-$\alpha_{1a}R$ heteromerization by using specific synthetic peptides corresponding to D_4R and $\alpha_{1a}R$ TM sequences. Functionally, we have observed in both heterodimers that the antagonist selective for one receptor is able to antagonize not only the signal mediated by its agonist but also the action mediated by the partner specific agonist (cross-antagonism). Also, the selective agonist for one receptor is able to decrease the signal mediated by the partner agonist (negative cross-talk) at the MAPK pathway level both in transfected cells and in rat tissue. Nevertheless, when intracellular calcium release and cAMP accumulation were analysed, these functional characteristics were only detected in the $D_{4.4}R$-$\alpha_{1a}R$ heteromer. These results suggest a functional relevance of the D_4R-α_{1a} R interaction, which seems to be modified in presence of the ADHD related $D_{4.7}R$ variant.

P.24. AMPAR-TARP STOICHIOMETRY DIFFERENTIALLY DETERMINES AMPAR BIOPHYSICAL PROPERTIES

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

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P.25. SNAP-25 PHOSPHORYLATION BY PKA IS ORCHESTRATED BY MUSCARINIC M_1 AND M_2 GPCR RECEPTORS AT THE NEUROMUSCULAR JUNCTION

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The synaptosomal-associated protein 25 (SNAP-25), one of the three SNARE proteins of the core fusion vesicle complex, regulates vesicle docking, priming and triggering of fast exocytosis of synapses. One pathway that could modulate SNAP-25 is muscarinic signaling through M_1 and M_2 subtype receptors, which are GPCR receptors that tune neurotransmission through their opposed signaling. At the adult neuromuscular synapse, M_1 increases end-plate potentials while M_2 decreases them. However, many points remain uncertain of how muscarinic signaling could influence SNARE machinery. The kinase PKA is a node where both pathways converge and its phosphorylation of SNAP-25 Thr138 could be a mechanism by which muscarinic receptors influence vesicle release. Accordingly, we studied the effects of subtype-selective and non-selective blockers of muscarinic signaling in the rat phrenic nerve-hemidiaphragm model. Results show that M_2 signaling inhibits M_1 and PKA in general by downregulating the PKA catalytic Cβ subunit, upregulating the regulatory RIIβ and translocating R subunits to the membrane. This inhibition also extends to SNAP-25 Thr138 phosphorylation. On the other hand, M_1 signaling downregulates RIIβ and translocates R subunits to the cytosol without affecting SNAP-25. Altogether, these results reveal that SNAP-25 Thr138 is orchestrated by the muscarinic receptor M_2 subtype and could be linked to its downregulation of neurotransmission in the neuromuscular junction.

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SENSORY AND MOTOR SYSTEMS

P.26. LOSS OF TRESK K^+ CHANNEL ENHANCES ACUTE AND CHRONIC ITCH

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

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P.27. TRESK BACKGROUND K+ CHANNEL REGULATES SENSORY NEURON EXCITABILITY AND CONTRIBUTES TO MECHANICAL AND COLD PAIN

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TRESK is a background K^+ channel highly expressed in spinal cord, dorsal root and trigeminal ganglia sensory neurons, where it has been involved in modulating sensory neuron excitability and firing. Changes in channel expression and function have been reported to enhance nociceptor excitability after injury or inflammation. To determine the role of TRESK in sensory transduction, we first compared the excitability and membrane properties of small/medium-sized sensory neurons in whole cell patch clamp recordings of cultured DRG neurons from wild type and TRESK knockout mice, which presented a reduced action potential threshold, increased membrane resistance and enhanced repetitive firing upon depolarization. Recordings of skin nociceptive fibers showed strong activation in response to cold in the absence of TRESK channel. In agreement, behavioral experiments in TRESK ko mice revealed a decreased mechanical threshold to von Frey hairs and an enhanced cold sensitivity. No significant changes were found for thermal sensitivity to warm or hot temperatures. Nocifensive behavior after capsaicin injection was unaltered while the response to AITC was slightly diminished. Interestingly, ko mice presented a reduced response to hypertonic and hypotonic stimuli even after sensitization with PGE_2. During inflammation, ko mice showed a decreased phase I response in the formalin test, while phase II was unaltered. In the CFA-induced inflammatory model, both mechanical and thermal sensitivity were enhanced compared to wt animals. Mechanical and thermal hyperalgesia were also enhanced in the sciatic nerve cuffing model of neuropathic pain. Finally, the oxaliplatin-induced cold sensitization was absent in ko mice. In summary, our results indicate that TRESK has a significant contribution regulating the excitability of certain populations of sensory neurons mainly involved in mechanical and cold pain sensing. Moreover, a down-regulation of its expression as occurs after nerve injury might contribute to the generation of the hyperalgesia and allodynia observed during chronic pain.

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P.28. PROTEOMIC QUANTITATIVE STUDY OF DORSAL ROOT GANGLIA AND SCIATIC NERVE IN TYPE 2 DIABETIC MICE

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The most common and debilitating complication of diabetes is peripheral neuropathy (DPN) affecting both sensorimotor and autonomic components of the peripheral nervous system (PNS). Diabetic patients present abnormal sensory perception which includes loss of pain and temperature feeling as well as burning sensation, skin tenderness, hyperalgesia and spontaneous pain. In more advanced stages foot ulcers and neuropathic deformities appear, which eventually result in 40% of the non-traumatic amputations. The pathology of DPN is characterized by sensory and motor axonal atrophy accompanied by demyelination and loss of nerve fibers, followed by abnormal regeneration. The pathogenesis of DPN is multi-factorial and a high number of disturbances appear to influence in the progression of the disease. Despite the economic burden and human costs, nowadays there is not a specific treatment to cure diabetic peripheral neuropathy. Complications of diabetes are poorly manifested in mouse models since they do not live long enough. A comparative study showed that the BKS-Db/Db mouse model, with a mutation in the leptin receptor gene, is one of the few mouse strains with T2DM including obesity, hyperinsulinemia and hyperglycemia, that develops DPN. The majority of previous research has applied transcriptomics tools such as RNA-microarrays or RNA-seq to study the deleterious effect of diabetes on dorsal root ganglia and sciatic nerve, however, to our knowledge, no prior studies have explored the role of T2DM on the nervous system proteome.

In this work, we have performed functional studies on BKS-Db/Db mice to evaluate their peripheral neuropathy development followed by a proteomic characterization of lumbar dorsal root ganglia and sciatic nerve after 15 weeks of diabetes by TMT labelling and LC/MS/MS analysis. Results are going to be discussed in the presentation along with the tools used for data validation. Our final aim is to detect new targets to finally develop advanced therapeutic strategies.

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P.29. STUDY OF THE PREVENTIVE EFFECTS OF A VEGETAL POLIFENOLIC EXTRACT OVER THE DEVELOPMENT OF CENTRAL NEUROPATHIC PAIN IN FEMALE SWISS MICE

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Neuropathic pain refers to pain caused by an injury or a disease that affects the nervous system and persists for months or years. This pain prevents people who suffer it to be able to do daily activities because stimuli that should not be painful are perceived as painful. What is sought is to improve the quality of life of people suffering from neuropathic pain but with current medications only half of the patients achieve a significant clinical reduction of pain and the best pharmacological combination only reduces pain in 30% of patients. In addition, these treatments cause multiple side effects, which is why it is being seek effective medications that totally reduce these effects. Currently, a research branch is about natural products being polyphenols an example of these. In this study, the effect of a new plant polyphenolic extract (EVP) was evaluated at 10 and 15 mg/kg concentrations in Swiss female mice with spinal cord injury (SCI). The treatments were administered just after the injury during 6 consecutive days. On days 0, 7, 14 and 21 after the operation (dpo) the painful sensory activity of the animals was evaluated by plantar test and by Von Frey test. In addition, at 21 dpo, histological analysis was carried out to study the levels of astrogliosis (GFAP) and microgliosis (Iba1). The obtained results show that the lowest dose (10 mg/kg) of the plant polyphenolic extract reduces thermal hyperalgesia, mechanical hyperalgesia, and reduces the reactivity of astrocytes and microglia. On the other hand, for the dose of 15 mg/kg the results obtained are not as good although the effect of this same extract could be evaluated, eliminating the exciting molecule of this type of plant to be able to arrive to the conclusion of if high doses of this extract are toxic or not.

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P.30. LONG-TERM FUNCTIONALITY OF TRANSVERSAL INTRANEURAL ELECTRODES IS IMPROVED BY DEXAMETHASONE TREATMENT

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Neuroprostheses aimed to restore lost functions after a limb amputation are based on the interaction with the nervous system by means of neural interfaces. Among the different designs, intraneural electrodes implanted in peripheral nerves represent a good strategy to stimulate nerve fibers to send sensory feedback and also to record nerve signals to control the prosthetic limb. However, intraneural electrodes, as any device implanted in the body, induce a foreign body reaction (FBR) that results in the tissue encapsulation of the device. The FBR causes a progressive decline of the electrode functionality over time due to the physical separation between the electrode active sites and the axons to interface. Modulation of the inflammatory response has arisen as a good strategy to reduce the FBR and maintain electrode functionality. In this work, transversal intraneural multi-channel electrodes (TIMEs) implanted in the rat sciatic nerve have been tested for 3 months to evaluate stimulation and recording capabilities under chronic administration of dexamethasone. Dexamethasone treatment significantly reduced the threshold for evoking muscle responses during the follow-up compared to saline-treated animals, without affecting the selectivity of stimulation. However, dexamethasone treatment did not improve the signal-to-noise ratio of the recorded neural signals. Dexamethasone treatment allowed to maintain more working active sites along time than saline treatment. Thus, systemic administration of dexamethasone appears as a useful treatment to improve the functionality of chronically implanted neural electrodes.

P.31. ANTI-HYPERALGESIC EFFECTS OF (-)-EPIGALLOCATECHIN-3-GALLATE TREATMENT IN TWO MODELS OF PATHOLOGIC PAIN

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Aims: The aim of the present work was to compare the anti-hyperalgesic effects of (-)-Epigallocatechin-3-gallate (EGCG) in two experimentally induced pathological pain models – reserpine-induced myalgia (RIM; fibromyalgia-like model) and spinal cord contusion (SCI) – during the acute phase.

Methods: For the first pain model, a set of CD1 female mice were subjected to several subcutaneous injections of reserpine (RIM) up to 23 days post-induction (dpi). Then, another set were subjected to spinal cord contusion (SCI) using the weight drop technique (2g; 25mm) to create the second pathological pain model. Moreover, mice were daily treated with EGCG (10, 15 or 20 mg/kg) during one week starting at 28 dpi in the RIM model or immediately after the surgery in the SCI model. Thermal hyperalgesia was assessed weekly by Hargreaves test until the end of the experimental period – up to 49 dpi or 21 dpo in RIM and SCI models respectively.

Results: Although EGCG exerted dose-dependent anti-hyperalgesic effects in both models, some differences were recorded. In the SCI model, while all the doses were able to prevent the thermal hyperalgesia up to 7 dpo, only 15 and 20 mg/kg showed preventive effects until the end of the experimental period. Regarding the RIM model, 20 mg/kg EGCG dose was the most effective, since exerted faster and longer-term effects up to 49 dpi when compared with 10 or 15 mg/kg doses.

Conclusions: EGCG has anti-hyperalgesic effects in both models. Thus, it may be suitable treatment to modulate hyperalgesia irrespective of its underlying origin, which may be as diverse as spinal cord injury or fibromyalgia.

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P.32. QUANTITATIVE ASSESSMENT OF THE TAIL-LIFT REFLEX TO MEASURE VESTIBULAR DYSFUNCTION IN RATS

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Loss of vestibular function results in loss of vestibulo-ocular and vestibulo-spinal reflexes, with distinct impacts on gaze and motor control. Quantitative assessment of these diverse disturbances in both humans and animals are necessary to link human dysfunction to cellular and molecular data derived from animal models. The aim of this study was to evaluate new quantitative measures to assess loss of vestibulo-spinal reflexes following ototoxic exposure in rats. The ototoxic compound 3,3'-iminodipropionitrile (IDPN) was used to cause vestibular loss in adult male Long-Evans rats in two different models of exposure: acute exposure (0, 400, 600 or 1000 mg/kg, single injection, i.p.) and chronic exposure (0 or 20 mM in the drinking water for 4 or 8 weeks). Before, during, and up to 13 weeks after exposure the tail-lift reflex and the air-righting reflex of the rats were video recorded for quantitative assessment. When lifted by the tail, healthy rats show a landing reflex consisting in forepaw and body axis extension, while vestibular deficits result in ventral bending responses. The animals were also evaluated by a previously validated behavioral test battery. Finally, the vestibular sensory epithelia were collected and processed for scanning electron microscopy and immunofluorescent analysis to estimate hair cell density. Acute IDPN caused loss of vestibular hair cells and behavioral deficits that showed no or only limited recovery. Chronic IDPN caused behavioral deficits with a degree of reversibility that related to the extent of the hair cell loss. The tail-neck-nose angle in the tail-lift reflex test showed high correlation with the data from the behavioral test battery, and related to hair cell loss. We conclude that the tail-lift test provides a simple quantitative measure of vestibulo-spinal dysfunction in rats.

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P.33. *EX VIVO* ELECTROMYOGRAPHIC STUDY OF SPONTANEOUS MUSCULAR ELECTRICAL ACTIVITY IN MICE

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Background: Spontaneous electrical activity (SEA) is a type of electromyographic record that can be obtained from relaxed healthy muscles. This SEA consists of end-plate noise (EPN) and end-plate spikes (EPS). Even though SEA has been studied since the 1950s, its origin in the spontaneous neurotransmission of the neuromuscular junction (NMJ) is not proven in a reliable way. The objective of this study is to assess the participation of NMJ in the generation of SEA.

Methods: Muscle areas were recorded by using electromyography at 1 mm and at 10 mm of the intramuscular nerves in ex vivo diaphragm. The presence of EPN and EPS in each area was recorded and their amplitude was calculated. The amplitude of EPN was also evaluated before and after a quick incubation with ClK (30 mM).

Results: The number of areas with EPN and EPS and their amplitude decrease progressively from near the nerve to 10mm away. Moreover, 10 mm apart from the intramuscular nerve, no EPN was recorded. Finally, 3 seconds after CIK exposure the EPN amplitude increases by 300%.

Conclusions: The spontaneous electrical activity recorded with electromyography is related to the spontaneous release of acetylcholine by NMJ.

P.34. STUDY OF HYPOTHALAMIC ENDOCANNABINOIDS FLUCTUATIONS WITH FAT-RICH DIETS: IMPORTANCE DURING THE DEVELOPMENT OF OBESITY IN MALE AND FEMALE MICE

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The endocannabinoid system plays a critical role in the central regulation of energy homeostasis. However, the effect of acute and chronic high fat diet (HFD) administration on endocannabinoids levels, its relationship to obesity development and the existence of a sexual dimorphism has not been studied. Our aim is to analyse the time-course of hypothalamic 2-arachidonoylglycerol (2-AG) and anandamide (AEA) levels in male and female mice during diet-induced obesity, and whether the differences observed correlate to changes in brown adipose tissue (BAT) thermogenesis and body weight. Male and female C57BL/6J mice were fed a standard diet (SD) or HFD for 7, 14, 28, 60 and 90-days. Body weight and food intake were controlled on the time-course of the experiment. BAT thermogenesis and 2-AG and AEA levels were analysed. Body weight progressively increased by exposure to HFD with similar tendency in both sexes. This data correlated with plasmatic leptin levels whereas food intake did not vary along treatment. BAT thermogenesis markers showed an activation peak of expression under 7 days of HFD followed by a decrease in their expression under prolonged HFD treatment. Female mice evidenced a higher activation of BAT thermogenesis under SD or HFD conditions compared to male mice. The analysis of hypothalamic endocannabinoids revealed a substantial increase of 2-AG and AEA levels after 7-days of HFD, which gradually decreased over time, reaching levels even lower than basal ones at 2-3 months of HFD. Female mice had higher levels of endocannabinoids in hypothalamus and plasma than male mice. Interestingly, the time-profile of hypothalamic endocannabinoids showed a negative correlation with plasmatic endocannabinoids and a positive correlation to the activation peak of BAT thermogenesis.

In this study, we postulate that the increase in hypothalamic endocannabinoids could be linked to the activation of BAT thermogenesis in response to short-term HFD to counteract obesity.

P.35. THERMAL HYPERALGESIA RESPONSES AND INTRAEPIDERMAL DENSITY OF CGRP-POSITIVE FIBERS IN TWO ANIMALS MODELS OF PATHOLOGICAL PAIN: A PRELIMINARY STUDY

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Skin punch biopsy testing has emerged as a diagnostic standard for small fiber neuropathy (SFN). Symptoms of SFN usually present distally, manifesting as foot or leg pain, and include paresthesia, allodynia, hyperesthesia, and numbness. Skin punch biopsy testing has emerged as a diagnostic standard for SFN. The European Federation of Neurological Societies recommended the measurement of the density of small fiber epidermal innervation in skin biopsy. Here, adult female ICR-CD1 mice were subjected to chronic constriction of right sciatic nerve (group CCI) and to spinal cord contusion (group SCI), two standard animal models of peripheral and central pathological pain, respectively. Control animals were subjected to surgery without injury of sciatic nerve (group Sham-CCI) and/or spinal cord (group Sham-SCI). Thermal hyperalgesia was evaluated by the plantar test, recording the withdrawal latency to a heat stimulus. Then animals were deeply anesthetized and the plantar pads were removed from the hind limbs, which were fixed in Zamboni solution for 14 days. Then Zamboni's solution was changed by a cryoprotective solution of 30% sucrose in PBS for 14 days. Distal plantar pads of right hind limbs were sectioned by a cryostat, and the histological sections were immunolabelled with CGRP for the visualization of peptidegic nociceptive fibers. The density of intraepidermal profiles was determined through the images captured from the histological sections. The results show that animals with CCI and SCI significantly remove the hind limbs before their respective controls, indicating that the nerve and / or spinal cord injury causes thermal hyperalgesia. Likewise, the density of intraepidermal profiles in the SCI animals was significantly higher than their respective controls, while no significant differences were observed in the CCI animals and their respective controls. These findings suggest that traumatic spinal cord injury causes changes in peripheral innervation.

MENTAL AND BEHAVIOR DISORDERS

P.36. PERIPUBERTAL STRESS ALTERS THE STRUCTURE AND THE CONNECTIVITY OF INTERNEURONS AND PYRAMIDAL NEURONS IN THE PREFRONTAL CORTEX ON MICE

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Stress exposure during early life, and particularly peripubertal stress, is an important risk factor for developing psychopathological disorders. It is known that chronic stress causes differential alterations in the structure of pyramidal neurons and interneurons in the adult medial prefrontal cortex (mPFC). However, little is known about how peripubertal stress affects excitatory and inhibitory neurons in the different regions of the prefrontal cortex. In the current study, we have subjected both female and male mice to an unpredictable peripubertal stress model and have analyzed its effects on inhibitory and excitatory networks, and on their plasticity. Interestingly, peripubertal stress causes a decrease in the expression of molecules related to inhibitory neurotransmission. To better understand how peripubertal stress affects inhibitory prefrontocortical networks, we have next studied the effects it has on the structure, connectivity of parvalbumin expressing interneurons and pyramidal neurons, as well as their perisomatic innervation (cannabinoid receptor 1+ or parvalbumin+) from basket interneurons. We have found that there is an increase in the complexity of the dendritic arbor of parvalbumin+ neurons in stressed female mice in the infralimbic and prelimbic regions. In addition, the density of cannabinoid receptor 1 expressing puncta around the somata of parvalbumin expressing interneurons and pyramidal neurons, was decreased in stressed females mice when compared to the stressed male group. Finally, we have seen some differences induced by stress in both sexes in the area of the somata of pyramidal cells and in the area of CBr1 expressing puncta surrounding them.

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P.37. PRESENCE OF $mGLU_5$ RECEPTOR IN THE PITUITARY GLAND

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Schizophrenia is a chronic illness that can be treated using antipsychotics. All of them block dopamine D_2-receptors. However, a considerable number of patients develop hyperprolactinemia due to D_2 blockade, which forces the change of doses or medication to avoid its adverse effects. The aim of this work is to study the posterior pituitary gland as it contains the cells responsible of prolactin production -lactotrophs- which also contain D_2 receptors. A way to reduce the side effects of antipsychotics could be based on the hypothesis that D_2 receptors can be allosterically modulated by other proteins like $mGlu_5$ receptors forming heterodimers with D_2 receptors [Cabello et al. J Neurochem. 2009 Jun;109(5):1497-507]. However, it is not known whether lactotrophs express $mGlu_5$ receptors. We have been able to determine the presence of $mGlu_5$ in the pituitary gland using immunohistochemistry and Western Blot assays with two different $mGlu_5$ antibodies. Our next step will be to determine the presence of $mGlu_5$ in specific cell types like lactotrophs and its colocalization with prolactin using immunohistochemistry. Next, we would study the protein-protein interaction between D_2-$mGlu_5$ using other techniques. With this investigation, we expect to obtain information useful to reduce the hyperprolactinemia induced by most antipsychotics and thus improve adherence to chronic treatment.

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P.38. COGNITIVE ABILITIES AND THE EXPRESSION OF CHOLINERGIC SIGNALING ARE MODULATED BY THE PESTICIDE CHLORPYRIFOS ACCORDING TO AGE AT EXPOSURE, SEX AND APOLIPOPROTEIN E (APOE) GENOTYPE IN TRANSGENIC MICE

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Recently, we have reported that the apolipoprotein E (*APOE*) genotype modulates cholinergic expression on forebrain in infant mice. In addition, apoE3 as well as apoE4 mice showed different short-term responses to postnatal exposure to the cholinergic pesticide, chlorpyrifos (CPF). However, long-term effects of this exposure according to APOE genotype and sex, on cognition and cholinergic expression have not yet been described. Moreover, the influence of the postnatal exposure on the response to an adult exposure to CPF has remained unclear. To address these research gaps, we exposed apoE3 and apoE4 transgenic mice of both sexes exposed to CPF at 0 or 1 mg/kg/day on postnatal days 10-15, and when adults, at 5 months of age mice were exposed to CPF at 0 or 2 mg/kg/day for 60 days at 5 months of age. At 6 months of age, mice were tested for spatial learning and memory in a Barnes maze. At the end of the adult exposure, mice were sacrificed and gene expression of cholinergic components was assessed in the hippocampus. We observed that apoE4 female mice had a poor performance in the spatial task, whereas postnatal exposure to CPF impaired spatial search strategies and spatial memory in apoE3 mice. In turn, adult exposure to CPF by itself ameliorated learning and memory abilities in apoE4 female mice. Concerning gene expression, choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) expression were increased in apoE4 mice. Postnatal exposure to CPF boosted ChAT mRNA levels in apoE4 mice, while adult exposure to CPF induced changes in acetylcholinesterase-S, α7- and α4-subunit nicotinic receptor expression in apoE4 female mice. The present findings provide new insights into *APOE*-dependent cholinergic signaling, which directly affects the response to CPF insult, especially in *APOE4* carriers.

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P.39. ASSESSMENT OF AUTISTIC-LIKE BEHAVIORS IN C57BL/6 MICE EXPOSED TO VALPROIC ACID AND APOE TRANSGENIC MICE

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Prenatal exposure to valproic acid (VPA), especially during the first trimester of pregnancy, resulted in a high prevalence of autism spectrum disorder (ASD) in the offspring. ASD is a heterogenous group of disorders characterized by abnormal communication and repetitive pattern of behavior. Some authors have associated the E4 isoform of the apolipoprotein E (APOE) gene with the disorder. The apoE protein competes with the Reelin protein for VLDLR and ApoER2 receptors, notably the apoE4 severely impair receptor recycling to the surface, leading to a Reelin resistance. Several studies reported an association between the Reelin gene and autism, for their importance in the neuronal migration during development. The aim of the present study is to assess in both male and female mice, autistic like-behaviors. C57BL/6 mice injected subcutaneously with a dose of 0 or 300 mg/kg/day on gestational days 12 and 13 were used as negative and positive control, respectively. ApoE transgenic homozygous mice carrying the human ε3 and ε4 alleles exposed to saline on gestational days 12 and 13 were also evaluated. During lactation, physical and neuromotor development were monitored and deficits in communication were assessed by means of ultrasonic vocalizations (USVs) analyses. Adolescent mice were also evaluated for social and repetitive behaviors. Our results showed transient delays in physical maturation in VPA-treated mice, while a diminution in USVs as well as altered social behavior were only observed in female mice prenatally exposed to VPA. Our findings suggested that VPA model can be of special relevance to study the disorder in females.

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NEURODEGENERATIVE DISEASES

P.40. MODULATION OF THE ENZYME SOLUBLE EPOXIDE HYDROLASE AS A THERAPEUTIC TARGET AGAINST NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases cursing with chronic inflammatory conditions represent a major health problem due to the global increase in life expectancy and age-related diseases. Thus, there is an urgent need for new approaches to mitigate neuroinflammation. Epoxyeicosatrienoic acids (EETs) are endogenous mediators that have several potentially protective functions including anti-inflammatory effects. EETs beneficial effects disappear when the enzyme soluble Epoxide Hydrolase (sEH) metabolizes them to the corresponding dihydroxyeicosatrienoic acids. Therefore, sEH enzyme is emerging as a promising pharmacological target, because its inhibition allows increasing EETs and keeping them active. The aim of the present study was to determine the half maximal inhibitory concentration (IC_{50}) of a newly synthetized sEH inhibitor (sEHi) series, agents A01, A02, A03, A04, A05 and A06, and study their toxicity and anti-inflammatory effects. Neuroblastoma SH-SY5Y cell cultures were used to the IC50 calculation and the safety level of each sEHi. To study the anti-inflammatory effects, lipopolysaccharide stimulated microglial-like BV2 cells were used to obtain conditioned media with the proinflammatory mediators. Modulatory effects on the inflammatory pathways iNOS and NFκB, and the release of cytokines were explored using techniques of Western Blot, immunocytochemistry and ELISA. All agents showed higher inhibitory potency than the reference compound 1-(1-propionylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU) and generally low cytotoxicity. They also showed promising anti-inflammatory properties. In this way, the newly synthesized sEHi agents could be a new therapy against the neuroinflammation present in neurodegenerative diseases as Alzheimer's disease, and in other neurological diseases with an inflammatory component.

Keywords: Soluble epoxide hydrolase, inhibitor, epoxyeicosatrienoic acids, neuroinflammation

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P.41. THE TRANSCRIPTION FACTOR C/EBPδ REPRESSES α-SYNUCLEIN TRANSCRIPTION: POTENTIAL PATHOGENIC EFFECTS OF C/EBPδ DEFICIENCY IN PARKINSON'S DISEASE

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α-Synuclein, one of the most abundant proteins in neuronal cytosol, plays an ill-defined role in neurotransmitter release and synaptic vesicle trafficking and is the main component of Lewy bodies, the intracellular protein aggregates that are considered the histological hallmark of Parkinson's disease. High α-synuclein levels are associated with increased risk for Parkinson's disease. Surprisingly little is known about the regulation of transcription of the human α-synuclein (SNCA) gene. CCAAT/enhancer binding protein δ (C/EBPδ) is a b-zip transcription factor expressed in the CNS that plays distinct roles in neurons and glial cells. C/EBPδ binding boxes are present in the SNCA genomic region, suggesting that this transcription factor could regulate SNCA transcription. The aim of this study was to determine if C/EBPδ regulates the expression of SNCA. We first observed that α-synuclein expression was markedly increased in C/EBPδ-deficient mice in several brain regions, both at mRNA and protein level. α-synuclein levels were also increased in C/EBPδ-deficient primary neuronal, but not glial, cultures. In accordance, C/EBPδ overexpression in neuroblastoma cells and in primary neuronal cultures markedly reduced α-synuclein expression. ChIP experiments demonstrated C/EBPδ binding to the SNCA genomic region of mice and humans. Finally, decreased C/EBPδ expression was observed in the substantia nigra and in iPSC-derived dopaminergic neurons from Parkinson patients resulting in a significant negative correlation between α-synuclein and C/EBPδ levels. This study demonstrates for the first time that C/EBPδ is a potent repressor of SNCA transcription. These findings suggest that reduced C/EBPδ neuronal levels could be a pathogenic factor in Parkinson's disease and other synucleinopathies and C/EBPδ activity a potential pharmacological target to treat these neurological disorders.

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P.42. OVEREXPRESSING α-SYNUCLEIN IN SEROTONIN NEURONS EVOKES DEPRESSIVE-LIKE BEHAVIORS IN MICE: REVERSAL BY SUSTAINED ADMINISTRATION OF ANTISENSE OLIGONUCLEOTIDES

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Anxiety and depression are frequently co-morbid with motor symptoms in Parkinson disease (PD). Hence, the lifetime risk of developing depression or anxiety is ≈60%, with a cross-sectional prevalence for each disorder being 30–40%. Recent studies report that anxiety and depression are associated with different patterns of PD-related factors, suggesting divergent underlying mechanisms. We studied the impact of a-synuclein overexpression in midbrain serotonin neurons (5-HT) on 5-HT neurotransmission and anxiety/depressive-like behaviors in mice. We used an adeno-associated virus vector (AAV5) which increased human-α-synuclein mRNA expression in raphe nuclei (290% of murine phenotype). Western-blot and immunohistochemical analysis revealed increased protein levels of human-α-synuclein, fibril α-synuclein and phospho-α-synuclein. Overexpression of α-synuclein in 5-HT neurons induced degenerative axonal changes, including a distorted appearance, as assessed in the hippocampus and caudate putamen (CPu) suggesting accumulation and aggregation of α-synuclein. Microdialysis experiments revealed reduced extracellular 5-HT concentrations in CPu of AAV5 mice in drug-evoked conditions (veratridine, citalopram and 8-OH-DPAT). Moreover, AAV5 mice displayed anxiety/depressive-like behaviors in dark-light box, tail suspension and forced swim tests. Finally, we examined whether reducing human α-synuclein overexpression selectively in 5-HT neurons could prevent pathological changes in the AAV5 model. Intracerebroventricular administration (i.c.v.) of an indatraline-conjugated antisense oligonucleotide targeting human α-synuclein (ASO1337, 100µg/day, 28 days) reduced human-α-synuclein mRNA and protein levels (-30% of sham mice) and reverted depressive-like behaviors. We concluded that α-synuclein pathology in 5-HT neurons is sufficient to impair emotional circuits and that conjugated ASO treatment would potentially prevent/delay the progression of PD non-motor symptoms.

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P.43. OVEREXPRESSION OF HUMAN WILD-TYPE OR MUTATED α-SYNUCLEIN OR LRRK2 IN MICE RESULTS IN DIFFERENTIAL DOPAMINERGIC NEUROTRANSMISSION, AND MOTOR, COGNITIVE AND EMOTIONAL BEHAVIORS

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The existing mouse models of Parkinson disease (PD) show important limitations to mimic this disorder. Most of the genetic mouse models recently developed failed to reproduce nigrostriatal dopaminergic (DA) neurodegeneration as well as motor and non-motor alterations. Here, we conducted a systematic analysis on nigrostriatal DA neurotransmission and on motor, emotional and cognitive behaviors using three mouse models of PD overexpressing human proteins. They included mutant transgenic mice: 1) *A30P*A53T α-synuclein (*A30P*A53T), 2) LRRK2*G2019S (LRRK2) and, 3) mice overexpressing wild-type α-synuclein in substantia nigra compacta (SNc) using a viral vector (AAV5). We evaluated the efficacy of an indatraline-conjugated antisense oligonucleotide (IND-ASO) to reduce α-synuclein expression in SNc of the three models. *A30P*A53T mice showed reduced veratridine-induced DA release (-51%) and increased amphetamine-induced DA concentration (+285%) in the striatum vs. control mice. They also exhibited severe alterations of motor behavior in the cylinder and open field tests. LRRK2 mice showed increased striatal DA concentration after local nomifensine infusion (+190%) together with cognitive deficits mainly in the novel object recognition test. AAV5 mice displayed reduced striatal DA levels after veratridine (-86%) and nomifensine (-54%) infusion. Further, quinpirole failed to reduce DA release. AAV5 mice displayed altered motor and anxiety-like behaviors as well as cognitive deficits. Intracerebroventricular IND-ASO administration (100µg/day, 28 days) reduced α-synuclein expression in *A30P*A53T and AAV5 mice. In conclusion, these PD models could be useful to mimic different stages of PD, providing a better understanding of the pathophysiology of the disease, being also useful to evaluate new therapeutic approaches.

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P.44. MODULATION OF CALCIUM-SENSORS ON N-METHYL ASPARTATE (NMDA) GLUTAMATE RECEPTORS IN NEURODEGENERATIVE DISEASES

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As life expectancy grows the number of patients suffering from neurodegenerative diseases increase. Currently, Alzheimer's disease (AD) dementia is the pathology with a higher incidence among the elderly. Unfortunately there is not any really efficacious treatment for either curing the disease or addressing the cognitive symptoms. The little pharmacological arsenal to combat the disease consists of acetylcholinesterase inhibitors and ligands of N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors.

It is well established that activation of NMDA receptors increase the levels of intracellular calcium, which in turn binds to calmodulin (CaM) to activate the CaM kinase II (CaMKII) and to induce MAPK phosphorylation. A first aim of this work was to look for interactions involving calcium-binding proteins other than CaM. We therefore tested NCS-1, calneuron-1 and caldendrin, which were selected because they may interact with and modulate the action of G-protein-coupled receptors, namely adenosine A2A and dopamine D2 receptors. To our knowledge no similar experiments have been performed to identify interactions between ionotropic glutamate receptors and calcium sensor proteins.

We here provide data proving a direct molecular interaction between NMDA and CaM, NCS-1 and calneuron-1 calcium sensors but not with caldendrin. The interaction was proved in primary cultures (neurons and microglia) of cortex and hippocampus by Proximity Ligation Assay (PLA). The transgenic $APP_{Sw,Ind}$ mice model of AD was used to demonstrate both that the expression of the NMDA receptor and the degree of interaction with the different calcium sensors were altered. Distorted NMDA function and abnormal calcium-mediated effects in cells of $APP_{Sw,Ind}$ mice should be taken into account to understand the mechanism of AD-related neurodegeneration.

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P.45. ASTROCYTES PLAY A KEY ROLE IN LAFORA DISEASE

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The term "polyglucosan bodies" (PGBs) refers to complex molecular aggregates composed of relatively large glucose polymers reaching diameters of tens of micrometers. Various forms of PGBs are linked to specific diseases or particular situations, including Lafora bodies in Lafora disease, corpora amylacea in aged human brain and PAS granules in aged mice brain. Corpora amylacea and PAS granules contain neo-epitopes that can be recognized by natural antibodies, but it is not known if these neo-epitopes are also present in Lafora bodies or other type of PGBs.

In order to characterize different types of PGBs and to study the presence of neo-epitopes on them, immunohistochemical staining procedures were performed on brain sections from a) malin knockout mice (malin^{KO}, a mouse model of Lafora disease), b) mice from senescence-accelerated mouse prone 8 (SAMP8) strain, with accelerated senescence, and c) protein targeting to glycogen-overexpressing (PTG^{OE}) mice, with enhanced glycogen synthase activity.

Unexpectedly, two types of PGBs were detected in malin^{KO} mice: one in neurons, localized in the perikarya, and corresponding to the expected neuronal Lafora bodies; and the other in astrocytic processes and containing neo-epitopes, thus making them equivalent to corpora amylacea or PAS granules. These PAS granules, but not the neuronal PGBs, were also detected in SAMP8 and PTG^{OE} animals. We also observed that, although not specific of Lafora disease, the formation of PAS granules was highly increased in malin^{KO} mice. Thus, the absence of malin triggers the formation of neuronal Lafora bodies but also enhances the formation of astrocytic PAS granules. These results suggest that astrocytes, against current belief, are involved in the pathogenesis of Lafora disease.

P.46. DIFFERENTIAL ACCUMULATION OF TAU PHOSPHORYLATED AT RESIDUES THR231, SER262 AND THR205 IN HIPPOCAMPAL INTERNEURONS AND ITS MODULATION BY TAU MUTATIONS (VLW) AND AMYLOID-β PEPTIDE

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β peptide (Aβ) and hyperphosphorylated Tau protein (P-Tau). Our recent data showed a differential accumulation of Tau protein phosphorylated at Thr231 in distinct hippocampal neurons in VLW mice, which overexpress mutated human Tau. Here we demonstrate that, in VLW mice, accumulation of human P-Tau in pyramidal cells induces murine pThr231 Tau in hippocampal interneurons. In addition, we demonstrate that Tau phosphorylated at Ser262 (pSer262) and Thr205 (pThr205) is present specifically in the soma of some hippocampal interneurons in WT mice. The analysis of VLW and J20 mice shows that the density of hippocampal interneurons accumulating pThr205 Tau is lower in VLW mice than in controls. In contrast, the density of interneurons accumulating pThr205 Tau in J20 mice is increased compared to controls in hippocampal regions with higher Aβ plaque load, suggesting that pThr205 Tau is induced by Aβ. No significant differences were found in the density of hippocampal interneurons positive for pSer262 Tau in VLW or J20 mice compared to control animals.

We also demonstrate that pSer262 and pThr205 Tau are present in the soma of some hippocampal interneurons containing Parvalbumin, Calbindin or Calretinin in control, VLW, and J20 mice. Moreover, our results reveal that some interneurons accumulate pSer262 and pThr205 Tau in control and AD human hippocampi. All this data points to a specific role of pSer262 and pThr205 Tau in the soma of hippocampal interneurons in control and pathological conditions.

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P.47. TAU PHOSPHORYLATION AND REELIN EXPRESSION IN HIPPOCAMPAL INTERNEURONS IN MICE MODELS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common cause of dementia. It is characterised by two pathological hallmarks, the accumulation of amyloid-β (Aβ) peptide and the aggregation of hyperphosphorylated Tau protein (P-Tau). Previous data of our group showed that Tau phosphorylated at residues Thr205 (pThr205) and Ser262 (pSer262) was accumulated in hippocampal interneurons in control animals, and also in J20 and VLW mice accumulating Aβ and P-Tau, respectively. These results suggested a possible physiological role of these P-Tau forms in the soma of interneurons, although they are considered pathological markers of AD. In addition, we demonstrated an induction of pThr205 Tau by Aβ and a possible repression by P-TauHere, we assess the distribution of pSer262 and pThr205 Tau in the hippocampus of JV animals, a new mouse model presenting both AD features: Aβ peptide and P-Tau accumulation. Our results revealed that the density of pThr205 Tau-positive interneurons in JV animals is similar to WT conditions, pointing to a compensatory effect of Aβ induction on P-Tau repression. Our data also suggested a synergic effect by Aβ and P-Tau in the regulation of pSer262 Tau, because the number of pSer262 Tau-positive cells was increased only in the presence of both histopathological alterations. Finally, we studied Reelin expression in control and pathological conditions. Our results indicated that Aβ reduces Reelin expression in hippocampal interneurons. This suggests a direct feedback between Aβ and Reelin, which is altered in AD and may cause an abnormal Tau phosphorylation in interneurons. The phosphorylation of pThr205 Tau is dependent on GSK3β and CDK5, which are activated by Aβ and inhibited by Reelin. Our data suggests that the reduction of Reelin induced by Aβ would cause a greater activation of GSK3 and CDK5, inducing the phosphorylation of Tau in the Thr205 residue.

P.48. HUNTINGTON DISEASE SKIN FIBROBLASTS YIELD POTENTIAL BIOMARKERS OF DISEASE PROGRESSION

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an unstable CAG repeat expansion in the *huntingtin* gene. Symptoms usually start in the middle age and include a triad of motor, cognitive and emotional abnormalities. Current therapeutics are limited to symptomatic treatments, so there is an urge to find new interventions that delay or even prevent disease progression. To validate these new treatments, it is essential to have objective and reliable measures. Hence, development of disease-specific biomarkers has never been more important. To this aim, we have proposed fibroblasts of HD patients as peripheral tissue samples that could be used to analyse possible biomarkers of disease progression as well as for drug screening. Although many pathogenic mechanisms have been proposed, some are more likely to be relevant than others for biomarker development. Several evidence suggests that metabolic impairment and transcriptional dysregulation play an essential role in HD. Therefore, we first monitored different mitochondrial processes and found that fibroblasts derived from premanifest and manifest patients present increased mitochondrial fission as well as increased glycolysis leading to higher ATP levels, with no changes in oxidative phosphorylation. In addition, our investigations have also identified several miRNAs with an altered expression pattern in HD fibroblasts, some of them also found to be altered in HD *postmortem* brain samples. Validation of selected deregulated miRNAs confirmed their role as biomarkers of HD, including miR6124, miR210, miR493 and miR127. Two of these miRNAs, miR210 and miR127, also play an important role in mitochondrial function and bioenergetics, linking the alterations observed in these miRNAs with the ones observed in cell bioenergetics of our HD fibroblasts. In view of these results, we propose that alterations in bioenergetics could be used for new drug screening while miRNAs could represent potential biomarkers of onset and progression of HD.

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P.49. ELUCIDATING THE COMMUNICATION BETWEEN NEURONS AND ASTROCYTES IN TEMPORAL LOBE EPPILEPSY: ROLE OF THE BDFN-TRKB PATHWAY

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Epilepsy is one of the most common and disabling neurological disorders. Previous studies suggested that brain-derived neurotrophic factor (BDNF) - tyrosine receptor kinase B (TrkB) activation promotes epileptogenesis, but the mechanisms underlying this process remain unknown. Although blocking BDNF-TrkB signalling has been proposed as an approach to treat status epilepticus (SE), it has also been shown that a global inhibition of this pathway can be deleterious as it is related to severe dementia-like syndromesHaving this in mind, the potential to cause a partial inhibition seems an attractive approach. Given the increasing evidence stating that astrocytes play an important role in temporal lobe epilepsy (TLE), we hypothesized that the inhibition of this pathway in a cell-specific manner could be a useful tool. To this aim, we used genetic tools to selectively overexpress or inhibit BDNF/TrkB in neurons or astrocytes both *in vitro* (4-aminopiridina model) and *in vivo* (pilocarpine model). The results showed that the increase in TrkB-T1 after SE-induction with pilocarpine can be prevented with our genetic approach in both cell types and this goes with an important reduction in neuronal cell death and hyperlocomotion. Additionally, we also found that BDNF from astrocytes specifically regulates the total number of activated neurons in an *in vitro* model of TLE.

In summary, our work suggests a communication between neurons and astrocytes during TLE. Thus, the obtained findings indicate that a modulation of the pathway in astrocytes could be an attractive method to reduce SE progression while decreasing side effects.

P.50. RESVERATROL UPREGULATES THE EXPRESSION OF ANTIOXIDANT GENES IN IMMORTALIZED AD LYMPHOCYTES

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and first cause of dementia all over the world. It is characterized by a progressive neuronal loss. The main risk factor for developing AD is the age. Major pathological hallmarks include extracellular deposits of amyloid protein and intracellular deposits of neurofibrillary tau protein. AD brain also shows alterations developed in aged brain such as oxidative stress and inflammation. The study used immortalized lymphocytes from patients of sporadic AD and age-matched healthy controls (HC) with the following aims: First, analyze gene expression of oxidative stress and aging-related pathways in AD and HC lymphoblast cell lines. Next, investigate the potential role of resveratrol as antiaging and protective agent through the induction of further gene expression changes. Finally, establish the different sensitivity between the human immortalized lymphocyte cell lines and the human neuroblastoma cell line SH-SY5Y to the hormetic agent resveratrol and to oxidative stress inductors. Real-time PCR showed that oxidative stress and aging-related genes were differentially expressed in AD lymphoblasts compared to HC lymphoblasts with increases in CASP1, TXNIP, VPS13C, GPX1, PRDX5, SOD2, and decrease in CCS expression. Resveratrol incubated for 18h at concentration of 10, 20 or 50µM induced a general upregulation of antioxidant and detoxifying genes such as: CAT, NFE2L2, GSTZ1, and CCS, in both HC and AD lymphoblasts. Resveratrol also induced the expression of the anti-aging mitochondrial sirtuin SIRT3. At the concentrations tested, resveratrol was not cytotoxic to lymphoblast or SH-SY5Y neuroblastoma cultures, although the later were more sensitive to oxidative injuries. This study supports that immortalized lymphocytes is a suitable cell system to analyze molecular alterations of pathways common to non-neural and neural cells. Furthermore, resveratrol upregulation of antioxidant genes may contribute to improve physiological processes in aging and AD.

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P.51. TRANSCRIPTION FACTOR EB OVEREXPRESSION DRIVES A NEUROTROPHIC EFFECT THAT NEUROPROTECTS AND NEURORESTORES DOPAMINERGIC NEURONS IN A MOUSE PARKINSON'S DISEASE MODEL

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Neurotrophic factor-based therapy stands as one of the most promising disease-modifying therapeutic approach for Parkinson's disease (PD). However, axonal impairment and downregulation of their receptors may account for the lack of therapeutic success in clinical trials. An alternative to delivering neurotrophic factors to overcome these hurdles is to directly activate the intracellular signaling pathways responsible for their effect. In this study, we demonstrate that Transcription Factor EB (TFEB) overexpression by means of an adeno-associated viral vector (AAV) in mice substantia nigra drives a previously unknown bona fide neurotrophic effect that mimicks RET-mediated effects and involves the activation of the MAPK1/3 and AKT pro-survival pathways, giving rise to cell growth, neurite outgrowth, increased protein synthesis, higher tyrosine hydroxylase levels and boosting the amounts of dopamine to be released in the striatum. In PD, neuronal dysfunction and atrophy accompanied by a loss of phenotype that involves tyrosine hydroxylase downregulation precede actual neuronal death. These neurons are still viable and are possible targets for neurorestorative therapies. We show that TFEB overexpression protects SNpc dopaminergic neurons and is capable of restoring their activity and phenotype in the MPTP mouse model of PD. In this scenario, TFEB overexpression completely protects dopaminergic neurons, both at the cell body level as well as striatal dopaminergic terminals, and counteracts the deleterious events that are particularly relevant to MPTP neurotoxicity and PD. It has been suggested that TFEB neuroprotective effect may be due to its capacity to boost the autophagy-lysosomal system for the clearance of protein aggregates. However, we demonstrate that knocking down the master transcriptional repressor of autophagy ZKSCAN3 by means of an AAV-shZKSCAN3 is not sufficient to protect dopaminergic neurons in the MPTP mouse model. Overall, our results suggest that TFEB activation is an alternative neuroprotective/neurorestorative strategy to neurotrophic factor-based therapies for PD and related disorders.

P.52. THE CD200R1 MICROGLIAL INHIBITORY RECEPTOR AS POTENTIAL TARGET TO CONTROL NEUROINFLAMMATION AND RESULTING NEURONAL DAMAGE IN THE MPTP MOUSE MODEL OF PARKINSON'S DISEASE

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Several contact-dependent and contact-independent inhibitory mechanisms contribute to maintain microglial cells in a quiescent/surveillant phenotype in the CNS in physiological conditions. The presence of chronic glial activation in the brain of patients with neurological diseases suggests that these inhibitory mechanisms have been overloaded. We focused our interest on the study of one of these mechanisms, the interaction between CD200 ligand (mainly neuronal, but also present in astrocytes) and CD200R1 receptor (microglial) interaction. Alterations in CD200 and CD200R1 expression have been described in Alzheimer's disease and multiple sclerosis postmortem brain, suggesting that this system is altered in pathological conditions. The aim of the present work was to study the temporal pattern of cerebral expression of CD200 and CD200R1 in the acute MPTP experimental mouse model of Parkinson's disease, in the context of the neuroinflammation and dopaminergic neurodegeneration induced by MPTP administration. We also determined the effect of the modulation of this system on the dopaminergic neurodegeneration. Administration of MPTP led to a gradual reduction in TH immunoreactivity in the striatum and SNpc starting 2 hours or 2 days after MPTP treatment, respectively. In both the striatum and SNpc, microglia (Iba-1 immunoreactivity) displayed a transient reactive phenotype from the first day of treatment. Similarly, transient astroglial reactivity (GFAP-immunoreactivity) is evident in the striatum and SNpc from the second day of treatment. We observed a transient increase in the expression of pro-inflammatory cytokines (IL-1b, IL-6, TNF-a) and enzymes (Cox-2) in striatum and SNpc, and interestingly, this pro-inflammatory response was accompanied by alterations in CD200-CD200R1 expression. The administration of a CD200R1 agonist resulted in the inhibition of the neurodegeneration observedCollectively, these findings provide evidence for a correlation between CD200-CD200R1 alterations, glial activation and neuronal loss. The potentiation of CD200R1 stimulation may be a potential approach to control neuroinflammation and neuronal death in Parkinson's disease.

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P.53. TRANSCRIPTIONAL CHANGES LINKED TO AGE-DEPENDENT NEUROMELANIN ACCUMULATION IN A NOVEL HUMANIZED MOUSE MODEL: RELEVANCE TO PARKINSON´S DISEASE AND BRAIN AGING

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Parkinson's disease (PD) is characterized by a selective and progressive loss of neurons that contain the dark brown pigment neuromelanin (NM), especially neurons from the substantia nigra (SN) and the locus coeruleus (LC), as well as, to a lesser extent, neurons from the ventral tegmental area (VTA) and the dorsal motor nucleus of the vagus nerve (DMNV). NM has long been suspected to act as a potential key factor involved in the selective neurodegeneration of PD, though the physiological and potentially pathological significance of NM intracellular accumulation remains unknown because, in contrast to humans, animal species commonly used as in vivo experimental models, such as rodents, lack this pigment. To overcome this major limitation we have recently generated a humanized transgenic mouse model that represents the first experimental in vivo model that recapitulates the production and age-dependent intracellular accumulation of NM seen in humans. Using this unique animal model, we will assess the biological implications of progressive NM accumulation in cellular functions by profiling the transcriptome of selectively isolated NM-containing neurons from SN, VTA, LC and DMNV brain regions at different ages, using laser capture microdissection (LCM). Then, to determine the relevance of these identified NM-linked biological pathways to humans, expression profiling will also be performed in LCM-isolated NM-containing neurons from human SN, VTA, LC and DMNV brain regions at different ages. This approach will enable the discovery of physiological pathways related to NM accumulation in the human aging brain and shed light on the still unknown potential contribution of NM to the neurodegenerative process in PD.

P.54. ADAPTIVE IMMUNE RESPONSE MEDIATED BY CYTOTOXIC T LYMPHOCYTES IS AN EARLY AND PROGRESSIVE EVENT IN PARKINSON'S DISEASE

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The major neuropathological hallmarks of Parkinson's disease (PD) are progressive loss of midbrain dopaminergic neurons, presence of Lewy bodies enriched with α-synuclein and chronic inflammation. Mounting evidence indicates that the adaptive immune system, together with the innate one, plays an important role in the neurodegenerative process. However, its role on the onset and progression of the disease is still controversial. The first evidence that the immune system is involved in the pathogenesis was the observation of activated microglia surrounding dopaminergic neurons. The presence of activated microglia appeared to correlate with the deposits of α-synuclein. Later on, it was reported an increase of the number of both CD4+ and CD8+ T cells in the substantia nigra *pars compacta* (SN*pc*) of PD patients. While some authors suggest that the infiltration of CD4+ Th1/Th17 lymphocytes contributes to neuronal death, others point to a cytotoxic death mediated by CD8+ lymphocytes. In any case, it is widely accepted that the adaptive immune system just contributes to the progression of the disease.

To shed light on the role of the adaptive immune response on the onset of PD we characterized in healthy controls, incidental Lewy Body Disease (iLBD) (considered to be a preclinical PD) and PD post-mortem SN*pc* the following parameters: 1) the loss of dopaminergic neurons, 2) CD4+ and CD8+ T cells recruitment, 3) microglia activation and 4) α-synucleinopathy. For the first time we have demonstrated that CD8+ T cell infiltration is an early event in PD pathology since we have observed an increase of their numbers in iLBD cases. We have also demonstrated that neuronal loss correlates with CD8+ T cell recruitment in PD, but we have not detected an increase of CD4+ T cells. Overall, our results suggest that CD8+ T cells mediated an early and chronic deleterious immune response in PD.

P.55. MONOMERIC C REACTIVE PROTEIN INDUCES SIGNALING PATHWAYS LEADING TO DEMENTIA IN MICE

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There is strong evidence that brain ischaemic processes increase the risk of suffering age-related dementia. Stroke may induce the development of vascular dementia, but also of Alzheimer's disease (AD) which is the leading cause of dementia worldwide. Neuroinflammation derived from ischaemic damage in the vessels and brain paremchima may be the causative of neurodegeneration. We have previously demonstrated that the monomeric C- reactive protein (mCRP) may provide a causative link between stroke-associated inflammation and dementia, as its injection into the hippocampus of wild type mice induced loss of memory within one month period [Slevin et al., 2015; Sci Rep 5:13281]. mCRP is a monomeric protein that comes from the dissociation of the pentraxin C-reactive protein (CRP) that remain chronically within the extracellular matrix of ischaemic tissue after stroke. Here we reproduced the mouse model of dementia by mCRP and extended the period post injection up to 6 months to further characterize the mCRP effects on cognition and general behavior. Mice of the transgenic AD strain 5XFAD were also tested for comparison. Treatment with mCRP induced lack of learning and memory at one, three and six months after bilateral injection into the hippocampus of the mice. We assayed the hippocampus tissue for gene expression and protein levels in a search of changes underlying cognitive loss and neurodegeneration. First results revealed lower activation of signaling pathways related to Arc and Egr1 early genes in mCRP mice but not in 5XFAD mice. Main changes in 5XFAD were related to oxidative stress and gliosis markers. We will pursue the molecular study to discern differential patterns between mCRP and 5XFAD mice. Characterizing the mechanisms of mCRP-induced dementia might contribute to decrease AD incidence in the elderly.

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P.56. ANTI-AGING MECHANISMS MAY PREVENT DEVELOPMENT OF ALZHEIMER'S DISEASE

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The progressive increase in life expectancy has led to an increase in the incidence of age-related diseases, including Alzheimer's disease (AD). Currently there are no effective treatments against AD, therefore, the search for preventive treatments is of great interest to achieve healthy aging. We hypothesize that the activation of anti-aging molecular pathways may induce a preventive effect of cellular resilience against the development of AD pathological processes. We evaluated the proposed anti-aging compounds resveratrol and melatonin by diet supplementation in 3xTg-AD and control healthy NoTg mice. Both compounds activated the neuroprotective and longevity SIRT1 pathway. The changes in 3xTg-AD hippocampus included decline of Aβ and tau pathology, decrease of inflammatory markers that suggests a protection against inflammaging, increase of proteolytic mechanism against aberrant proteins, and beneficial changes in mitochondrial function and plasticity processes. Remarkably, these treatments induced upregulation of neuroprotective mechanisms in the healthy NoTg mice. Both resveratrol and melatonin protected against memory loss in 3xTg-AD and induced improved behavior in the healthy mice. Our data confirmed the potential of both anti-aging compounds to induce neuroprotection against AD-pathology, and unveiled their preventive effects by increasing brain resilience.

Keywords: Alzheimer disease, resveratrol, melatonin, resilience, neuroprotection, anti-aging.

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P.57. SPECIFIC EXPRESSION OF GDNF IN MUSCLES AS GENE THERPY STRATEGY FOR ALS

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Glial cell line-derived neurotrophic factor (GDNF) is a powerful growth factor that can protect motoneurons in vitro and in vivo. However, systemic administration of GDNF is associated with side effects like decreased weight and reduced activity. One of the aims in gene therapy is indeed, the restriction of transgene expression to the target tissues to avoid secondary effects. Nevertheless, treatment of certain pathologies, such as neuromuscular diseases may need both, specific but generalized, muscle or motoneuron transduction. To specifically target most skeletal muscles in the superoxide dismutase 1 mouse bearing the human G93A mutation (SOD1G93A), a model for amyotrophic lateral sclerosis (ALS), we intravenously injected AAV2/8 and AAV2/9 serotypes, coding for the luciferase reporter under the control of the muscle specific human desmin promoter, to wild-type and SOD1 mice. Animals were analyzed 5 weeks later by in vivo imaging. AAV2/8 was chosen due to a slighter higher specific expression in the heart and skeletal muscles (legs, arms, tongue, and diaphragm) independent of genotype and sex. Ex-vivo luciferase activity in tissue homogenates was negative in lung, spinal cord, brain and liver although, particularly in the liver, viral genome copies per cell were extremely high, as expected. AAV8-DesGDNF was administered in SOD1 and wild-type mice before the onset of the disease as a preventive therapeutic strategy. Muscle-specific GDNF expression correlated with preservation of functional tests from 12 weeks of age, assayed by ectrophysiological analysis suggesting a delayed disease onset in the SOD1 animals treated with GDNF. Motoneuron survival in treated SOD1 animals was also observed, closely related to the activation of the PI3k/Akt/ERK signaling pathways. Moreover, no adverse secondary effects were detected, highlighting the potential of this strategy for ALS gene therapy.

P.58. EFFECT OF THE ADENOSINE A_1 RECEPTOR G279S MUTATION IN ADENOSINERGIC SIGNALING: IMPLICATIONS FOR PARKINSON'S DISEASE

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Early-onset Parkinson disease (EOPD), defined by age of onset between 20 and 40 years of age, accounts for 4-10% of all Parkinson disease (PD) patients [1]. Recent epidemiologic studies have indicated a more readily evident genetic component in EOPD in contrast to late-onset idiopathic PD [2]. The neuropathological dominant symptoms of both EOPD and PD include the death of substantia nigra neurons, thus causing striatal dopamine deficiency, and the presence of intracellular inclusions known as Lewy bodies. In a recent study, a new mutation (c.835G>A) in the coding sequence of the adenosine A_1 receptor (*i.e.* A_1R^{G279S}) has been linked to the development of EOPD [3]. The A_1R is a G protein-coupled adenosine receptor widely expressed within the brain (i.e. hippocampus, frontal cortex, thalamic nuclei and basal ganglia) which shows a neuroprotective function. Accordingly, we recreated the *ADORA1* 8835G>A mutation (c.835G>A) *in vitro* and the receptor functional consequences were assessed in heterologous expression systems. To this end, the cDNA encoding A_1R^{WT} and A_1R^{G279S} was transfected in HEK-293 cells and the expression levels, ligand binding properties and
cAMP accumulation in response to agonist incubation was determined. Interestingly, while the A_1R ligand binding properties were not affected by the mutation, A_1R^{G279S} expression was significantly increased when compared to the wild type receptor. Subsequently, the ability to inhibit adenylate cyclase upon receptor activation was evaluated through cAMP determinations. Interestingly, we observed that A_1R^{G279S} was unable to reduce forskolin-induced cAMP accumulation, thus suggesting that the G279S mutation precluded receptor function (*i.e.* protein G_i activation). Overall, our results shed light into the role of A_1R function in PD in general and its particular contribution to EOPD, thus leading to potential therapeutic strategies to manage parkinsonism.

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P.59. HUMAN KLOTHO AS A BIOMARKER AND THERAPEUTIC MOLECULE FOR ALZHEIMER'S DISEASE

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Klotho (KL) protein is considered a neuroprotective factor and an ageing suppressor, since its mutation causes systemic ageing and shortened lifespan in mice. KL deficiency is also involved in neuronal degeneration in hippocampus, memory deficits and increased oxidative stress in brain. On the contrary, KL overexpression is related to increased lifespan, improved memory and cognitive capacities, and reduced oxidative stress. Our group observed that the membrane (mKL) and the secreted (sKL) variants of KL had a different expression profile in mouse brain, and that sKL decreased more rapidly with age in Alzheimer's disease (AD) mouse model. sKL protein levels were also lower in AD mice compared to age-matched controls. Thus, it seems that KL could have an important role in AD. In this work, the gene expression of both mKL and sKL variants was analysed in brain of human AD patients and adults without neuropathological lesions. We observed different expression levels of sKL in each disease group and brain area. Besides, mKL was almost undetectable, suggesting that sKL is the most important KL variant in human brain. In addition, KL protein was measured in cerebrospinal fluid (CSF) of AD patients. Soluble KL was clearly reduced in CSF of moderate AD patients compared to controls with no cognitive declineThese results suggest that KL, and especially the sKL variant, could serve as a diagnosis biomarker for AD. Moreover, the potential of KL as therapeutic molecule should be explored

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P.60. EXPRESSION ANALYSIS OF AGING-SUPPRESSOR FACTORS IN CORTEX AND HIPPOCAMPUS FROM MOUSE MODELS OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases are defined as conditions that generate a progressive degeneration and loss of function of the nervous system, characterised by specific pathophysiology and medical prognosis. Study of proteins with aging-suppressor or neuroprotective properties in the brain of model animals for the study of neuronal diseases can lead to the discovery of altered pathways, which could be restored after a specific gene therapy treatment in the brainαKlotho (KL) is a gene expressed mainly in proximal tubules in the kidneys and choroid plexus of the brain. It is composed by 5 exons, presenting two major splicing variants: a long one (m-KL) composed by two similar extracellular domains (KL1 and KL2), and a short one (s-KL), containing just KL1 domain and a short 5'end specific sequence. Klotho anti-aging properties were discovered when mutations reducing this gene expression generated an early-aging phenotype in mice. Expression of other genes implied in aging-suppression events was also studied, both implied in Klotho signalling pathway, like FOXO3 or PPARγ, or acting through other yet unknown mechanisms. Both s-KL and m-KL transcripts were analysed to detect possible changes specifically in one of the isoforms, which could be disease-specific. Gene expression was studied in cortex and hippocampus samples obtained from mouse models for the study of different neurodegenerative diseases. Expression of different genes was seen to be altered mainly in hippocampus of the studied mouse models, whereas Klotho isoforms and FOXO3 presented higher changes in the cortex area. Some of the expression changes could be related to the symptoms observed in these mouse models, and could also be explained as a compensatory mechanisms to decrease stress generated by the pathology.

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P61. SOLUBLE X45 FACTOR GENE THERAPY WITH ADENO-ASSOCIATED VECTORS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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In the immunopathogenesis of multiple sclerosis (MS) are involved both innate and adaptative immunity. Within the last one, different cell lines have been identified as triggers of autoreactive and inflammatory response. In order for these cell lines to differentite and expand, several key molecules are necessary, and one of them is the X45 ligand (X45L). In this way, X45L knockout mice show a decreased Experimental Autoimmune Encephalomyelitis (EAE) clinical progression and a reduction in the number of autoreactive and proinflammatory lymphocytes.

The main objective of this project is to block the X45L by a soluble blocking factor of X45L (X45F) by its intravenous administration in an adeno-associated vector of serotype 8 (AAV8) with a 5·10^11 viral genomes/animal dose 21 days before the EAE induction by MOG_{40-55} peptide mice immunization. Subsequently, daily evaluation of animals was done to establish the degree of physical affectation (clinical score) and weight loss.

We demonstrated by inmunofluorescence that cells infected with an adenovirus codifying the X45 factor, the molecule show a cytoplasmic localization but it was not observed in cytoplasmatic membrane, suggesting it acts as a soluble protein. In addition, we detected the protein in the culture medium of these cells, verifying its secretability. We also demonstrated the functionality of the molecule by analyzing X45F capacity to block murine X45L dependent Stat3 phosphorylation in splenocytes in vitro.

Finally, when the molecule was tested in vivo in 2 independent experiments, the treament with AAV8-X45F significantly improves the clinical score, the incidence and the histological affectation of the disease compared to the AAV8-Null treated group in the EAE mouse model.

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P.62. TRANSGENERATIONAL EPIGENETIC INHERITANCE OF RESVERATROL DIET PREVENTS COGNITIVE IMPAIRMENT THROUGH EPIGENETIC CHANGES AND OXIDATIVE STRESS IN OFFSPRING OF SAMP8 MICE MODEL

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While the elderly segment of the population continues to grow in size and importance, dementia incidence increases exponentially. Lifestyle factors such as diet, exercise, education, among others, influence ageing progression. In particular, Central Nervous System (CNS) can benefit from a diet strategies to prevent signs of senescence, as cognitive decline or neurodegenerative diseases, like Alzheimer's disease (AD). Recent evidence has shown that epigenetic modifications can occur in response to environmental stimuli, on of the most important of which is diet. The mechanisms by which diet affects epigenetics are not fully understood. Dietary polyphenols such as resveratrol possess anti-oxidant, anti-aging, neuroprotection, anti-inflammatory, anti-diabetic, anti-dementia, and extends lifespan. Resveratrol has pleiotropic effects, demonstrating its activity through several biological pathways, including epigenetics. The aim of the work is to study the influence of an enriched resveratrol diet in maternal offsprings. We evaluated cognitive effects of dietary resveratrol on inheritance in 6-month-old Senescence-accelerated mouse prone 8 (SAMP8) mice. We found a reduction in cognitive impairment by Novel Object Recognition Test (NORT) in F1 and F2. At the molecular level, we observed a reduction antioxidant enzymes gene expression such as Hmox1, Aldh2, Nrf2 as well as hydrogen peroxide levels (H2O2) in the hippocampus of both generations. Besides, a reduction of ER stress proteins and epigenetic changes in global DNA methylation (5-mC) and hydroxymethylation levels (5-hmC), as well as modifications in histone H3 and H4 acetylation levels were found across generations. Likewise, we found changes in the hippocampal gene expression of several chromatin-modifying enzymes, such as Hdac2, G9a, Dnmt1, Dnmt3a/b. These new findings suggest that the environmental influence by early-diet can modify the risk of cognitive decline and provide a better understanding of the mechanisms involved in neurodegeneration.

P.63. ENDOPLASMIC RETICULUM STRESS MEDIATED NEUROTOXICITY IS PREVENTED IN JNK1 AND JNK3 KNOCK-OUT MICE TREATED WITH KAINIC ACID

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Kainic acid (KA) has been used to establish excitotoxicity in in vivo models. The KA treatment induces neuronal death in brain, particularly in hippocampus, linked to mitochondrial dysfunction and endoplasmic reticulum stress (ER). The ER stress is a vital mechanism for the homeostatic control of the cell. However, prolonged ER stress induces cell death that can be mediated through the activation of c-Jun N-terminal kinases (JNKs). JNKs are members of the MAPK (Mitogen-Activated Protein kinase) that, in mammals, are encoded by three genes: Mapk8 (Jnk1), Mapk9 (Jnk2), and Mapk10 (Jnk3), which are expressed differentially in the brain. The aim of this study was to analyse the role of the different JNK isoforms in the ER stress induction and their link with neuronal death, after KA treatment. To achieve this goal, we used JNK KO mice (jnk1$^{-/-}$, jnk2$^{-/-}$ and jnk3$^{-/-}$) and wild type mice (WT), at basal conditions and after intraperitoneal injections of KA. In each condition, we identified several adaptive and pro-apoptotic ER stress targets, such as ER-luminal-binding-protein (BiP), C/EBP-homologous-protein (CHOP) and protein kinase-like endoplasmic reticulum kinase (PERK). The results revealed that ER stress pathway was down regulated in jnk1$^{-/-}$ and jnk3$^{-/-}$ mice, compared with WT, although they had differential response. Moreover, we observed that the activation of pro-apoptotic intrinsic pathway was prevented, since the levels of Bax, a pro-apoptotic protein, decreased together with the reduction of caspase activity. Thus, the lack of JNK1 and JNK3 prevents the induction of ER stress and consequently the reduction of intrinsic apoptotic pathway. All together, these findings justify the development of selective inhibitors for JNK1 and JNK3 in order to develop drugs with neuroprotective effects.

P.64. EXPLORING THE ELUSIVE COMPOSITION OF CORPORA AMYLACEA OF HUMAN BRAIN

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Corpora amylacea (CA) are polyglucosan bodies that accumulate in the human brain during the ageing process and are also present in large numbers in several neurodegenerative conditions. Theories regarding the function of these structures are regularly updated as new components are described. However, in previous work we pointed out a specific methodological problem related to CA immunohistochemical studies, and that problem generate inconsistences in the description of their composition. In order to verify the presence on CA of some previously described components, and to determine the presence of certain potential components that have not been studied to date, immunohistochemical staining procedures were performed on human brain sections from Alzheimer's disease and non-Alzheimer's disease donors.We show that, contrary to previous descriptions, CA do not contain GFAP, S100, AQP4, NeuN or class III β-tubulin, and we questioned the presence of other previously described CA components. However, we observed that CA contains ubiquitin and p62, both of them associated with processes of elimination of waste substances, and also glycogen synthase, an indispensable enzyme for polyglucosan formation.

The study shows that it is imperative to continue reviewing previous studies ant theories about CA and, especially, reinforce the vision of CA as waste containers in which deleterious or residual products are isolated for later elimination through the action of the innate immune system.

P.65. HIGHLY MOTILE AND MIGRATING MICROGLIA AND TUMOR-ASSOCIATED MACROPHAGES DENSELY POPULATE PSEUDO-PALISADES IN GLIOBLASTOMA

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

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

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