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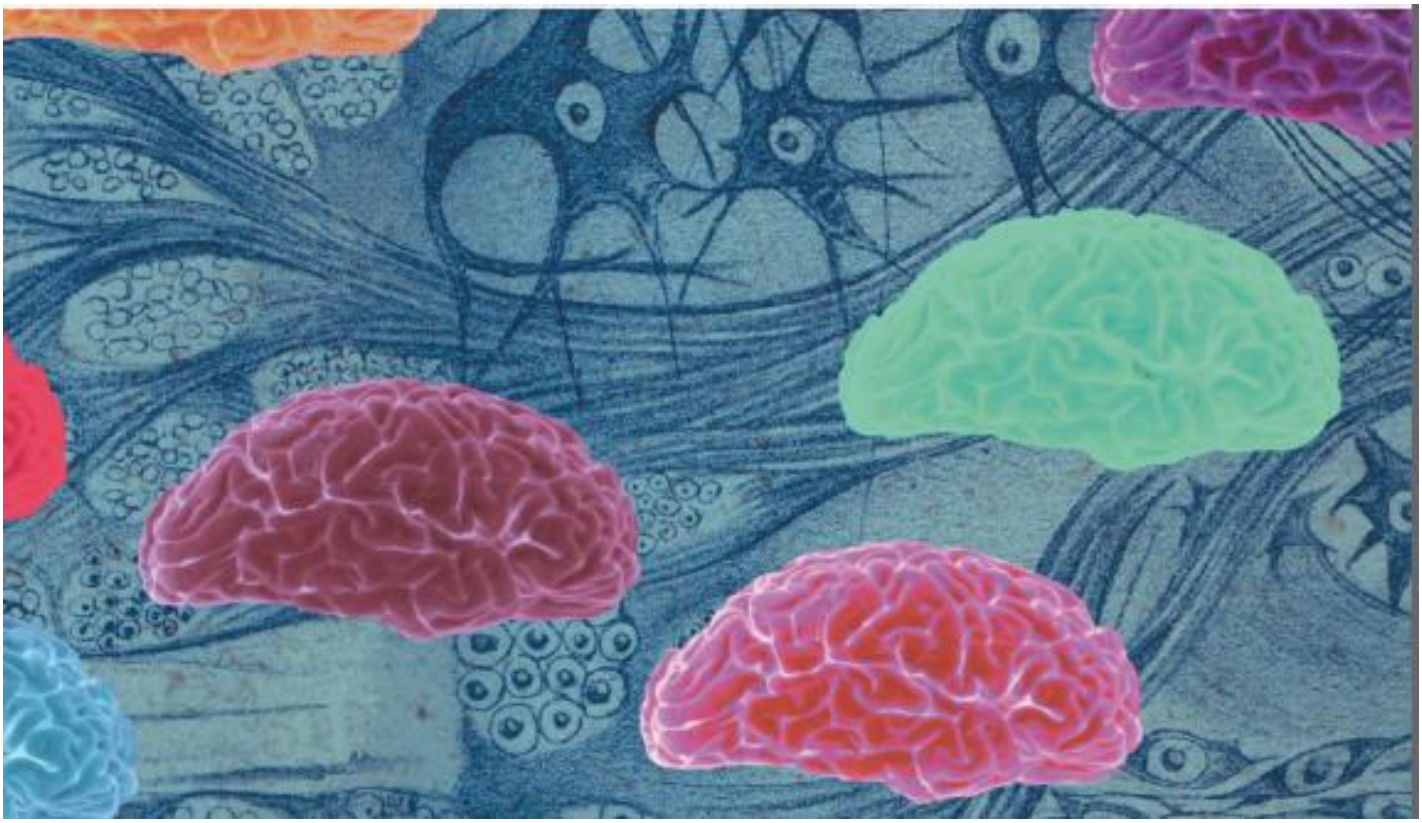
Abstracts Book



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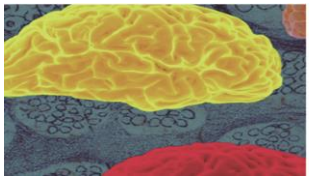
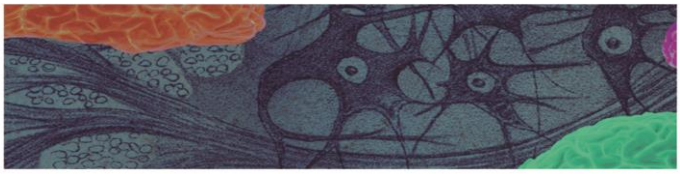
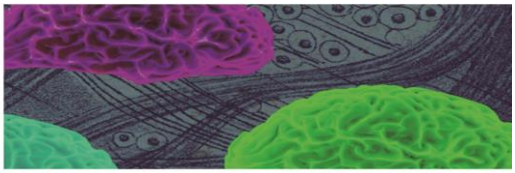
COVER PICTURE:

"Brain frills" ("Florituras cerebrales")

Artistic rendering that combines the idea of the existence of multiple neurotransmitters (coloured brains) with the action of these neurotransmitters through the brain cells..

(Javier de Felipe)

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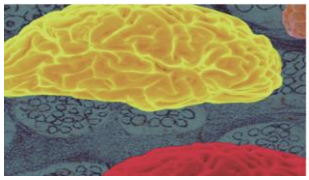

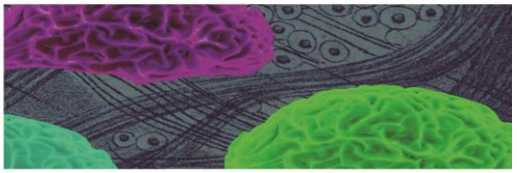

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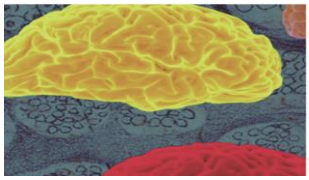
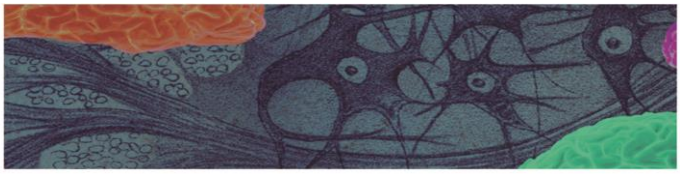
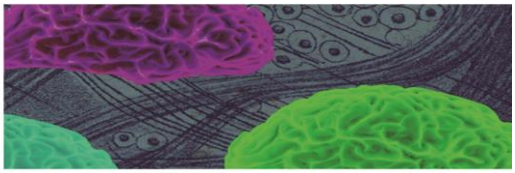

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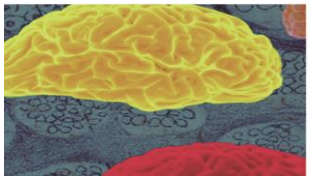

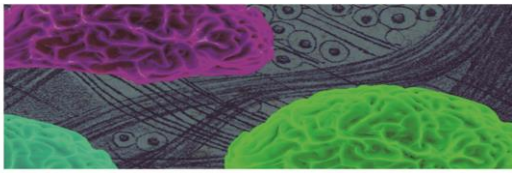

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POSTER 1

Principal Investigator: Cuadrado Pastor, Antonio (CIBERNED, Madrid).

Title: **NRF2 deficiency replicates transcriptomic changes in Alzheimer's patients and worsen APP and TAU pathology.**

Authors: Rojo AI, Pajares M, García-Yagüe AJ, Lovestone S , Ribe E, Nevado A , Buendía I , López MG, van Leuven F, Yamamoto M, Cuadrado A.

Abstract: Failure to translate successful neuroprotective preclinical data to a clinical setting in Alzheimer's disease (AD) indicates that amyloidopathy and tauopathy alone provide an incomplete view of disease. We have tested here the relevance of additional homeostatic deviations that result from loss of activity of transcription factor NRF2, a crucial regulator of multiple stress responses whose activity declines with ageing. A transcriptomic analysis demonstrated that NRF2-KO mouse brains reproduce 7 and 10 of the most dysregulated pathways of human ageing and AD brains, respectively. Then, we generated a mouse that combines amyloidopathy and tauopathy with either wild type (AT-NRF2-WT) or NRF2-deficiency (AT-NRF2-KO). Deficiency in NRF2 worsen pathological AD hallmarks including oxidative stress, neuroinflammation, APP and TAU pathology. To determine whether pharmacological targeting of transcription factor NRF2 might provide a disease-modifying therapy, we employed dimethyl fumarate (DMF), a drug already in use for the treatment of multiple sclerosis. Daily oral gavage of DMF during 8-weeks improved memory, cognition and motor problems in the AT-NRF2-WT mice compared with the vehicle treated animals. This study demonstrates the relevance of normal homeostatic responses that decline with ageing, such as NRF2 activity, and provide a new strategy to fight AD.

POSTER 2

Principal Investigator: Cuadrado Pastor, Antonio (CIBERNED, Madrid).

Title: **Pharmacologic targeting of GSK-3 and NRF2 provides neuroprotection in a preclinical model of tauopathy.**

Authors: Cuadrado A, Kügler S, Lastres-Becker I.

Abstract: Tauopathies are a group of neurodegenerative disorders where TAU protein is presented as aggregates or is abnormally phosphorylated, leading to alterations of axonal transport, neuronal death and neuroinflammation. Currently, there is no treatment to slow progression of these diseases. Here, we have investigated whether dimethyl fumarate (DMF), an inducer of the transcription factor NRF2, could mitigate tauopathy in a mouse model. Methods: Neuronal, astroglial and microglial cell lines were treated with DMF to determine if the NRF2 transcriptional signature is cell type-dependent. The signaling pathways modulated by DMF were also studied in mouse embryonic fibroblast (MEFs) from wild type or KEAP1-deficient mice. The effect of DMF on neurodegeneration, astrocyte and microglial activation was examined in Nrf2+/+ and Nrf2?/? mice stereotaxically injected in the right hippocampus with an adeno-associated vector expressing human TAUP301L and treated daily with DMF (100 mg/kg, i.g) during three weeks. Results: DMF induces the NRF2 transcriptional signature in all brain-derived cell types through a mechanism that involves KEAP1 but also PI3K/AKT/GSK-3-dependent pathways. DMF modulates GSK-3? activity in mouse hippocampi. Furthermore, DMF modulates TAU phosphorylation, neuronal impairment measured by calbindin-D28K and BDNF expression, and inflammatory processes involved in astrogliosis, microgliosis and pro-inflammatory cytokines production. Interpretation: This study reveals neuroprotective effects of DMF beyond disruption of the KEAP1/NRF2 axis by inhibiting GSK3 in a mouse model of tauopathy and supports repurposing of this drug for treatment of these diseases.

POSTER 3

Principal Investigator: Cuadrado Pastor, Antonio (CIBERNED, Madrid).

Title: **Role of the transcription factor NRF2 in hippocampal neurogenesis and in a mouse model of Alzheimer´s Disease.**

Authors: Robledinos-Antón N , Rojo AI, Ferreiro E, Núñez A, Krause K, Jaquet V, Cuadrado A.

Abstract: Neural stem/progenitor cells (NSPCs) located at the subgranular zone (SGZ) of the hippocampus, differentiate into granular neurons, which in turn integrate in circuits that participate in learning and memory functions. Considering that oxidative stress, neuroinflammation and proteinopathy alter the activity of the SGZ neurogenic niche, in this study we hypothesized that Nuclear Factor-Erythroid 2-Related Factor 2 (NRF2), a master regulator of homeostatic responses, might modulate the fate of NSPCs at the SGZ. We have produced genetically modified mice that express human mutated forms of TAU(P301L) and the amyloid protein precursor APP(V717I) in the presence or absence of NRF2. We report that parental Nrf2-/- and APP/TAU/NRF2-deficient mice have altered synaptic plasticity and cognition as they present a decrease in hippocampal long term potentiation and poor performance in the Morris water maze test. At the neuropathological level, we found an accelerated loss of NSPCs pool and neuronal differentiation during mouse ageing from 3 to 12 months. In SGZ-derived neurospheres the clonogenic and proliferative capacity of NSPCs was severely reduced in Nrf2-/- mice compared to Nrf2+/+, and the neuron/astroglia ratio was reduced. This effect was reversed by transducing Nrf2-/- NSPCs with a lentiviral vector expressing NRF2. Our findings demonstrate the importance of NRF2 in the maintenance of proper proliferation and differentiation rates of hippocampal NSPCs. Pharmacological interventions aimed at up-regulating NRF2 may preserve the neurogenic functionality of the hippocampus and improve cognitive functions in AD.

POSTER 4

Principal Investigator: Fuentes Rodríguez, José Manuel (CIBERNED, Cáceres).

Title: **Protein acetylation in fibroblasts from Parkinson's disease patients.**

Authors: Yakhine-Diop Sokhna MS, Rodríguez-Arribas M, Martínez-Chacón G, Uribe-Carretero E, Sánchez-Molinero L, Gómez-Sánchez R, Bravo-San Pedro JM, Aiastui A, López de Munain A, Niso-Santano M, González-Polo RA, Fuentes JM.

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Several environmental and genetic factors have been implicated in the pathogenesis of PD. The most prevalent genetic cause of PD are mutations in LRRK2 gene, and the G2019S LRRK2 variant appears in 5-6% of familial PD and 1-2% in sporadic cases. This disorder is generally characterized by appearance of aging-related features, i.e. mitochondrial dysfunction, oxidative stress and, lately, neuronal death. Despite of all those common hallmarks, the molecular phenotype differs between sporadic and genetic models of PD. Protein acetylation level is enhanced in G2019S LRRK2 fibroblasts; however, it is decreased in the idiopathic PD. This difference is due to the activation of AMPK? signaling pathway in the pathogenic mutation of LRRK2. Moreover, inhibition of this pathway by Dorsomorphin improves the mitochondrial membrane potential. However, inhibition of class I and II histone deacetylases (HDACs) or class III HDACs by Trichostatin A (TSA) and Nicotinamide (NAM), respectively, exacerbates apoptotic cell death in both PD models. Upregulation of AMPK inhibits acetyl-CoA carboxylase (ACC), which increases production of acetyl-CoA and, subsequently, acetylation of protein levels. This study highlights the role of AMPK? in neurodegenerative diseases through the regulation of acetylated proteins and the decrease of mitochondrial membrane potential. M. N-S was supported by "Contrato Juan de la Cierva" (JCI-2012-14383) from Ministerio de Economia y Competitividad, Spain. M. R-A. was supported by a FPU predoctoral fellowship (FPU13/01237) from Ministerio de Educación, Cultura y Deporte, Spain. R. G-S. was supported by a Marie Sklodowska-Curie Individual Fellowship (IF-EF) from the European Commission. J.M. B-S. P. was funded by La Ligue Contre le Cancer. J. M. F. received research support from the Instituto de Salud Carlos III, CIBERNED (CB06/05/004) and Instituto de Salud Carlos III, FIS, (PI15/00034). R. A. G-P. was supported by a "Contrato destinado a la retención y atracción del talento investigador, TA13009" from Junta de Extremadura, as well as research support from the Instituto de Salud Carlos III, FIS, (PI14/00170). This work is cofounded by Fondo Europeo de Desarrollo Regional (FEDER). The authors also thank FUNDESALUD for helpful assistance.

POSTER 5

Principal Investigator: Pérez Castillo, Ana María (CIBERNED, Madrid).

Title: **CCAAT/Enhancer binding protein beta silencing mitigates glial activation and neurodegeneration in a rat model of Parkinson's disease.**

Authors: Morales-Garcia JA, Gine E, Hernández-Encinas E, Aguilar-Morante D, Sierra-Magro A, Van Bulck M, Sanz-SanCristóbal M, Alonso-Gil S, Sánchez-Lanzas R, Castaño JG, Santos A, Pérez-Castillo AM.

Abstract: The CCAAT/Enhancer binding protein beta (C/EBPbeta) is a transcription factor involved in numerous physiological as well as pathological conditions in the brain. However, little is known regarding its possible role in neurodegenerative disorders. We have previously shown that C/EBPbeta regulates the expression of genes involved in inflammatory processes and brain injury. Here, we have analyzed the effects of C/EBPbeta interference in dopaminergic cell death and glial activation in the 6-hydroxydopamine model of Parkinson's disease. Our results showed that lentivirus-mediated C/EBPbeta deprivation conferred marked in vitro and in vivo neuroprotection of dopaminergic cells concomitant with a significant attenuation of the level of the inflammatory response and glial activation. Additionally, C/EBPbeta interference diminished the induction of alfa-synuclein in the substantia nigra pars compacta of animals injected with 6-hydroxydopamine. Taking together, these results reveal an essential function for C/EBPbeta in the pathways leading to inflammatory-mediated brain damage and suggest novel roles for C/EBPbeta in neurodegenerative diseases, specifically in Parkinson's disease, opening the door for new therapeutic interventions.

POSTER 6

Principal Investigator: Iglesias Vacas, Teresa (CIBERNED, Madrid).

Title: **Decreased novel Kidins220 Splice Isoform in Huntington´s Disease.**

Authors: Sebastián-Serrano A, Belmonte-Alfaro A, Pose-Utrilla J, García-Guerra L, Del Puerto AM, Santos M, Schiavo G, Lucas JJ, Iglesias T.

Abstract: Huntington´s disease (HD) is a hereditary neurodegenerative disorder characterized by brain atrophy particularly in striatum leading to personality changes, chorea and dementia. Kinase D interacting substrate (Kidins220) is a neurotrophin effector and is spatially and temporally regulated in the brain. We previously demonstrated that Kidins220 presented altered levels in others neurodegenerative diseases like Alzheimer´s disease (AD). Specifically, Kidins220 is augmented in necropsies from AD patients where it accumulates with hyperphosphorylated tau. Regarding HD, nothing is known about the expression and role of Kidins220. Here we have explored the levels of Kidins220 full-length and splicing variants in the striatum and other less affected brain areas from HD patients and R6/1 mice. We have found a dramatic decrease in the recently identified alternative terminal exon splice isoform of Kidins220 (Kidins220 C2) in the striatum of HD patients with similar result in the HD mouse model R6/1, even at early symptomatic age. Immunofluorescence analyses revealed neurons as the cellular lineage that express higher levels of Kidins220 C2. These results demonstrating diminished levels of Kidins220 C2 in the striatum of HD patients together with an early decrease of this protein in the brain of R6/1 mice points to a possible novel contribution of this specific isoform to HD pathogenesis.

POSTER 7

Principal Investigator: Del Río Fernández, José Antonio (CIBERNED, Barcelona).

Title: **iPS Cell Cultures from a Gerstmann-Sträussler-Scheinker Patient with the Y218N PRNP Mutation Recapitulate tauopathy.**

Authors: Matamoros-Angles A, Gayosso LM, Richaud-Patin Y, di Domenico A, Vergara C, Hervera A, Sousa A, Fernández-Borges N, Consiglio A, Gavín R, López de Maturana R, Ferrer I, López de Munain A, Raya Á, Castilla J, Sánchez-Pernaute R, Del Río JA.

Abstract: Gerstmann-Sträussler-Scheinker (GSS) syndrome is a fatal autosomal dominant neurodegenerative prionopathy clinically characterized by ataxia, spastic paraparesis, extrapyramidal signs and dementia. In some GSS familiar cases carrying point mutations in the PRNP gene, patients also showed comorbid tauopathy leading to mixed pathologies. In this study we developed an induced pluripotent stem (iPS) cell model derived from fibroblasts of a GSS patient harboring the Y218N PRNP mutation, as well as an age-matched healthy control. This particular PRNP mutation is unique with very few described cases. One of the cases presented neurofibrillary degeneration with relevant Tau hyperphosphorylation. Y218N iPS-derived cultures showed relevant astrogliosis, increased phospho-Tau, altered microtubule-associated transport and cell death. However, they failed to generate proteinase K-resistant prion. In this study we set out to test, for the first time, whether iPS cell-derived neurons could be used to investigate the appearance of disease-related phenotypes (i.e, tauopathy) identified in the GSS patient.

POSTER 8

Principal Investigator: Del Río Fernández, José Antonio (CIBERNED, Barcelona).

Title: **Cellular Prion Protein is a new receptor for ?-Synuclein in Neurons.**

Authors: Urrea L, Segura-Feliu M, Masuda-Suzukake M, Hervera A, Pedraz L, García Aznar JM, Vila M, Samitier J, Torrents E, Hagesawa M, Gavín R, Ferrer I, Del Río JA.

Abstract: The cellular prion protein, encoded by the gene Prnp, has been reported to be a receptor of ?-amyloid. Their interaction is mandatory for neurotoxic effects of ?-amyloid oligomers. In this study, we aimed to explore whether the cellular prion protein participates in the spreading of ?-synuclein. Results demonstrate that Prnp expression is not mandatory for ?-synuclein spreading. However, although the pathological spreading of ?-synuclein can take place in the absence of Prnp, ?-synuclein expanded faster in PrPC-overexpressing mice. In addition, ?-synuclein binds strongly on PrPC-expressing cells, suggesting a role in modulating the effect of ?-synuclein fibrils.

POSTER 9

Principal Investigator: Canela Campos, Enric Isidre (CIBERNED, Barcelona).

Title: **Dopamine receptor ligands as potent ?2-adrenoceptor ligands.**

Authors: Canela EI, Casadó-Anguera V, Sánchez-Soto M, Moreno E, Bender E, Yano H, Cai N-S, Meiler J, Cortés A, Ferré S, Casadó V.

Abstract: Dopamine (DA) acts on specific receptors belonging to the G protein-coupled receptor (GPCR) family and are categorized in two main groups including D1-like and D2-like receptors. The G?i/o-coupled dopamine D2-like receptor family comprises three subtypes: the D2 receptor, D3 receptor, and D4 receptor. The ?2 adrenoceptors are also GPCRs associated with inhibitory G-proteins. It has been classified in mammals into three highly homologous but distinct subtypes including ?2A, ?2B, and ?2C. It has long been recognized that several brain areas show a mismatch between the innervation by the catecholamines norepinephrine and dopamine and the density of their canonical receptors. Particularly notorious is the low norepinephrine innervation and relative high density of Gi/o-coupled ?2A and ?2C adrenoceptors in the striatum, major target for the ascending dopaminergic system. This prompted the possibility that dopamine could provide an effective endogenous ligand for both adrenoceptors, but previous studies were inconclusive. In the present study, we addressed the same question by analyzing the ability of dopamine and several putative selective dopamine receptor ligands to bind and activate ?2A and ?2C adrenoceptors in transfected mammalian cells using sensitive bioluminescent resonance energy transfer-based techniques that detect ligand-dependent activation of specific G proteins or activation of their effector adenylyl cyclase. Furthermore, we also analyzed their ability to bind to ?2 adrenoceptors in cortical tissue, which predominantly expresses ?2A adrenoceptors, and striatal tissue, which expresses both ?2A and ?2C adrenoceptors. Binding events were further studied with computer modeling of ligand docking. The results not only provide conclusive evidence for ?2A and ?2C adrenoceptors being both norepinephrine and dopamine receptors, but also for being common targets for compounds previously characterized as Gi/o-coupled dopamine receptor ligands. Furthermore, the present study demonstrates the G?i/o protein subtype is fundamental in the determination of the unique pharmacological profile of Gi/o-coupled catecholamine receptor ligands.

POSTER 10

Principal Investigator: Canela Campos, Enric Isidre (CIBERNED, Barcelona).

Title: **Antiinflammatory and neuroprotective effects of cannabinoids in neurodegenerative diseases.**

Authors: Navarro G, Reyes I, Angelats E, Etayo I, Borroto-Escuela D, Fuxe K, Canela EI, Franco R.

Abstract: Modulation of the levels of the endocannabinoid 2-arachidonoyl-glycerol by inhibiting monoacylglycerol lipase alters glial phenotypes and provides neuroprotection in a mouse model of Parkinson's disease. The fatty acid amide hydrolase inhibitor, URB597, administered chronically to mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid (MPTPp) over 5weeks prevented MPTPp induced motor impairment but it did not preserve the dopamine levels in the nigrostriatal pathway. The symptomatic relief of URB597 was confirmed in haloperidol-induced catalepsy assays, where its anti-cataleptic effects were both blocked by antagonists of the two cannabinoid receptors (CB1 and CB2), and abolished in animals deficient in these receptors. These results demonstrated an effect of fatty acid amide hydrolase inhibition on the motor symptoms of Parkinson's disease in two distinct experimental models that is mediated by cannabinoid receptors in both neurons and glia (Celorrio et al., 2016). The hypothesis of direct interactions between pairs of G-protein-coupled receptors relevant for CNS function, launched by Luigi Agnati and Kjell Fuxe, has been confirmed and is now widely accepted. Natural and synthetic cannabinoids target two types of G-protein-coupled receptors (GPCRs). Cannabinoid CB1 receptors, which are enriched in the CNS and cannabinoid CB2 receptors that are more abundant in peripheral tissues. Despite the moderate expression of CB2 receptors in brain, it has been demonstrated that CB1 and CB2 may for receptor heteromers (RHets) in the CNS. Therefore, natural or synthetic cannabinoids may act on CB1, CB2 and CB1 or CB2 containing heteromers. The research presented in this paper was undertaken to know whether these two receptors may be expressed in activated microglia. It is worth noting that current knowledge assumed that CB1 is more a neuronal than glial receptor whereas the opposite occurs for CB2. On the one hand, the expression of receptors and RHets is different in resting and activated microglia. Activation was assayed in the N9 cell line and in primary cultures of microglia using LPS and interferon gamma. On the other hand, the increase in CB1-CB2 RHets correlates with a potentiation of the effects of selective CB2 receptors. Our results show that the composition of cannabinoid receptors and RHets in resting microglia prevent microglia activation while in conditions of microgliosis due to Parkinsonian conditions, cannabinoid agonist regulate microglial activation. The results indicate that pharmacological manipulation of cannabinoid receptors in conditions of neuroinflammation may have relevant benefits in conditions of neuroinflammation. Celorrio M, Fernández-Suárez D, Rojo-Bustamante E, Echeverry-Alzate V, Ramírez MJ, Hillard CJ, López-Moreno JA, Maldonado R, Oyarzábal J, Franco R, Aymerich MS. Fatty acid amide hydrolase inhibition for the symptomatic relief of Parkinson's disease. Brain Behav Immun. 2016 Oct;57:94-105.

POSTER 11

Principal Investigator: Canela Campos, Enric Isidre (CIBERNED, Barcelona).

Title: **Adenosine A1-dopamine D1 receptor heteromers in spinal cord motoneurons.**

Authors: Moreno E, Rivera-Oliver M S, Mallol J, Cortés A, Ferré S, Díaz-Ríos M, Casadó V, Canela EI.

Abstract: Monoamines such as dopamine are an important group of neuromodulators that are released in the spinal cord circuits and are critical for the motor performance. At the spinal level, dopamine's motor excitatory effects are mainly mediated via a descendent dopamine system mostly controlled by the dopamine receptors of the D1 receptor subtype (D1R). Adenosine is a ubiquitous neuromodulator in the central nervous system, which is involved in numerous functions. More general functions include the regulation of arousal and its role in neuroprotection. Still general but circuit specific, adenosine plays a very significant role in the modulation of dopaminergic transmission, with implications for psychomotor activity and reinforcement. The modulatory role of adenosine on dopaminergic transmission depends largely on the existence of antagonistic interactions mediated by specific subtypes of adenosine and dopamine receptors, the so-called A1R-dopamine D1R interactions. It has been recently found a functional antagonistic interaction between A1R and D1R ligands in the mouse spinal cord that mediates the ability of caffeine to enhance locomotor-related activity by acting on spinal circuits, although the molecular mechanisms and cellular localization remained to be determined. In the present study, A1R-D1R heteromerization is first demonstrated in mammalian transfected cells using biophysical techniques such as bioluminiscent resonance energy transfer and bimolecular fluorescense complementation. We have also determined the specific localization of A1R-D1R heteromers in mouse spinal motoneurons through immunohistochemistry and with the use of a proximity ligation assay using antibodies directed toward the A1R and the D1R. In motoneurons, A1R-D1R heteromers mediate the modulatory control by adenosine and dopamine and the strong spinal pharmacological effects of caffeine and of A1R antagonists. These results can have important implications for the pharmacotherapy of spinal cord injury (SCI). Our hypothesis is that A1R-D1R receptor heteromers can be novel therapeutic targets for SCI and that the simultaneous administration of D1R agonists and A1R antagonists, by interacting with A1R-D1R heteromers, can prevent the SCI-induced motor alterations.

POSTER 12

Principal Investigator: Canela Campos, Enric Isidre (CIBERNED, Barcelona).

Title: **Cannabidiol is an allosteric modulator of cannabinoid CB2 receptors.**

Authors: Franco R, Martínez-Pinilla E, Varani K, Reyes-Resina I, Angelats E, Vincenzi F, Ferreiro-Vera C, Canela EI, Lanciego JL, Nadal X, Navarro G, Boreaos PA.

Abstract: The mechanism of action of cannabidiol (CBD), the main non-psychotropic component of Cannabis sativa L., is not completely understood. First assumed that the compound was acting via cannabinoid CB2 receptors (CB2Rs) it is now suggested that it interacts with serotonin 5-HT1A receptors; however, CBD does not bind with high affinity to neither cannabinoid nor serotonin receptors. In order to search for the possibility that CBD acts as an allosteric ligand of CB2R, both a radioligand and non-radioactive homogeneous binding assays were used in CB2R-expressing HEK-293T cells. Furthermore, to check whether CBD modulates the binding and action of CB2R agonists, intracellular cAMP levels were determined. Using membrane preparations from HEK-293T cells expressing the human version of the CB2R, we have confirmed that CBD does not bind with high affinity to the orthosteric site of the human CB2R where the synthetic cannabinoid, [3H]-WIN 55,212-2, binds. The natural component of marijuana was, however, able to negatively modulate the effect of the selective CB2R agonist, JWH133, on forskolin-induced intracellular cAMP levels. These results, that suggest an allosteric mode of action, were consistent with a CBD-mediated decrease in the dissociation kinetics of fluorophore-conjugated CB2R-selective compound, CM-157, detected by an homogenous time-resolved fluorescence resonance energy transfer (HTRF) binding assay. In conclusion, some of the actions of CBD may be mediated by interaction with an allosteric site in CB2R that leads to fine tuning modulation of the binding and effect of receptor agonists. Keywords Endocannabinoid, allosterism, G-protein-coupled receptor, phytocannabinoids, TR-FRET.

POSTER 13

Principal Investigator: Lanciego Pérez, José Luis (CIBERNED, Pamplona).

Title: **Gene therapy with beta-glucocerebrosidase for the treatment of Parkinson's disease and related synucleinopathies. Crossroads between Gaucher's and Parkinson's diseases.**

Authors: Sucunza D, Pignataro D, Rodriguez-Perez AI, Roda E, Dopeso-Reyes IG, Rico AJ, Labandeira-Garcia JL, Lanciego JL.

Abstract: Here we summarize our ongoing efforts focused on increasing glucocerebrosidase (GBA) activity to reduce alpha-synuclein burden in non-human primates. In an attempt to model a Parkinson's disease-like synucleinopathy, neurospecific AAV9 vectors coding for alpha-synuclein have been injected into the substantia nigra in a cohort of six macaques. Throughout a follow-up of six months, animals are being evaluated for UPDRS scores and microPET scans at regular intervals, later followed by histopathological analysis. Preliminary results showed an ongoing, progressive synucleinopathy with a follow-up of 3 months. These time-course studies are required to elucidate the best therapeutic window for AAV-mediated enhancement of GBA activity. Finally, the baseline GBA expression levels in control macaques have also been investigated by means of the immunohistochemical detection of GBA throughout the entire macaque brain. Although as with other lysosomal enzymes GBA was ubiquitously found in all neurons, a number of specific neuronal phenotypes expressing very high GBA levels were observed and characterized. In this regard, cholinergic neurons from the nucleus basalis of Meynert, dopaminergic neurons in the substantia nigra pars compacta, serotoninergic neurons in the raphe nuclei and noradrenergic neurons in the locus ceruleus were the ones showing the highest GBA baseline expression levels. Supported by ERC Advanced Grant number 340527 ReproPARK.

POSTER 14

Principal Investigator: Moratalla Villalba, Rosario (CIBERNED, Madrid).

Title: **The N370S-GBA1 mutation causes lysosomal cholesterol accumulation in Parkinson's disease.**

Authors: García-Sanz P, Orgaz L, Bueno-Gil G, Espadas I, Rodríguez-Traver E, Kulisevsky J, Gutiérrez A, Dávila JC, González-Polo RA, Fuentes JM, Vicario C,. Moratalla R.

Abstract: Background Heterozygous mutations in the GBA1 gene, which encodes the lysosomal enzyme ?-glucocerebrosidase-1 (GCase1), increase the risk of developing Parkinson's Disease (PD), although the underlying mechanisms remain unclear. The aim of this study is to explore the impact of the N370S-GBA1 mutation on cellular homeostasis and vulnerability in a patient-specific cellular model of PD. Methods We isolated fibroblasts from four PD patients carrying the N370S/wt GBA1 mutation and six controls to study the autophagy-lysosome pathway, endoplasmic reticulum (ER) stress and Golgi apparatus (GA) structure by Western blot, immunofluorescence, LysoTracker® and Filipin stainings, qRT-PCR and electron microscopy. We evaluated the impact on cell vulnerability by apoptosis, reactive oxygen species (ROS) and mitochondrial membrane potential with flow cytometry. Results The N370S mutation produced a significant reduction of GCase1 protein and enzyme activity and GCase1 retention within the ER, which interrupted its traffic to the lysosome. This led to ER stress activation and triggered unfolded protein response and GA fragmentation. Furthermore, these alterations resulted in autophagosome and p62/SQSTM1 accumulation. This impaired autophagy was due to dysfunctional lysosomes indicated by multilamellar body accumulation probably caused by increased cholesterol, enlarged lysosomal mass and reduced enzyme activity. This phenotype impaired the removal of damaged mitochondria, ROS production and enhanced cell death. Conclusions Our results support a connection between the loss of GCase1 function, cholesterol accumulation and the disruption of cellular homeostasis in PD. Our work reveals new insights into the cellular pathways underlying PD pathogenesis, providing evidence that GBA1-associated PD shares common features with lipid-storage diseases.

POSTER 15

Principal Investigator: Obeso Inchausti, José Ángel (CIBERNED, Madrid).

Title: **Calretinin and tyrosine hydroxylase interneurons in the striatum of pre-symptomaticand symptomatic MPTP monkeys.**

Authors: López-González N, Blesa J, García-Esparcia P, Trigo-Damas I, Cavada C, Ferrer I, Obeso JA .

Abstract: In Parkinson's disease (PD) the degeneration of the nigrostriatal dopaminergic projection has been shown to affect not only the morphological features of striatal projection neurons but also various types of striatal interneurons. Classically, the striatal interneurons are grouped into two main classes: the neurons that use acetylcholine as a neurotransmitter, and the gabaergic (GABA) neurons. Of all types of striatal interneurons, those expressing calretinin (CR-ir) are the most abundant in human and nonhuman primates, while those expressing tyrosine hydroxylase (TH-ir) are relatively few. The number of TH-ir and CR-ir neurons has been shown to increase with dopaminergic depletion in animal models of PD. However, the effect of levodopa (L-DOPA) treatment and degree of dopamine (DA) striatal denervation as conditioning factors of the number of these neurons is unclear. Here, we provide a detailed account of the morphological characteristics, topographical distribution and numerical densities of these two types of interneurons in monkeys rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication with different degrees of nigrostriatal lesion. We also analyze the effect of L-DOPA. Thus, we have been able to ascertain striatal TH-ir and CR-ir neurons at different stages of the pre-symptomatic and symptomatic parkinsonian condition. Our results show that the number of TH-ir cells increases very early in the evolution of nigrostriatal DA deficit in monkeys, including the stage when no motor signs are present. L-DOPA treatment abolished the numerical increase of TH-ir cells. While the small and medium size CR+ neurons did not change, the number of large CR+ neurons was also increased in parkinsonian monkeys. These data support the hypothesis that DA concentrations could regulate the numerical density and/or modulate the phenotypic expression of some striatal neurons, which could perhaps serve as an early compensatory mechanism in the pre-symptomatic stages of parkinsonism and be crucial to the pathophysiology of PD.

POSTER 17

Principal Investigator: Labandeira García, José Luis (CIBERNED, Santiago de Compostela).

Title: **Expression of angiotensinogen and receptors for angiotensin and prorenin in the rat and monkey striatal neurons and glial cells.**

Authors: Garrido-Gil P, Rodríguez-Pérez AI, Fernández-Rodríguez P, Lanciego JL, Labandeira-Garcia JL.

Abstract: The renin-angiotensin system (RAS) was initially considered as a circulating humoral system, which function is the regulation of blood pressure. However, it is now known that there exist local RAS in many tissues, including brain. In recent studies, we have demonstrated the presence of a local RAS in the substantia nigra of rodents and primates that modulates dopamine release and dopamine receptor expression. However, overactivation of local RAS exacerbates neuroinflammation, oxidative stress and dopaminergic cell death. In the striatum, it is not clear whether angiotensin receptors are located in dopaminergic terminals, glial cells and/or the projection neurons. The present study shows the location of major components of the RAS in striatal projection neurons of rats and monkeys (both in neurons of the direct and the indirect pathways). Striatal astrocytes and microglial cells also express major RAS components, which increase after induction of neuroinflammation by intrastriatal injection of lipopolysaccharide. Angiotensin receptors were located at the cell surface and also at cytoplasmic and nuclear levels. The results obtained by immunolabeling and confocal microscopy were confirmed with laser microdissection of striatal neurons and glial cells and detection of mRNA expression by PCR. The sequence of the resulting PCR products was verified by DNA sequencing. In addition to the interaction between angiotensin and dopamine receptors in dopaminergic neurons to regulate dopamine release, interaction between angiotensin and dopamine receptors in projection striatal neurons may further modulate the effects of dopamine on the direct and indirect pathways by fine tuning striatal dopaminergic neurotransmission.

POSTER 18

Principal Investigator: Labandeira García, José Luis (CIBERNED, Santiago de Compostela).

Title: **Interaction between the enteric dopamine and angiotensin systems. Effects of aging.**

Authors: Garrido-Gil P, Domínguez-Meijide A, Moratalla R, Guerra MJ, Labandeira-García JL.

Abstract: Gastrointestinal dysfunction is a common problem in the elderly. Aging-related changes in interactions between local dopaminergic and renin-angiotensin systems (RAS) have been observed in the brain, renal and vascular tissues. However, it is not known if these interactions also occur in the gut, and are dysregulated with aging. We showed a mutual regulation between the colonic dopaminergic system and RAS using young and aged mice deficient for major angiotensin and dopamine receptors. Aged rats showed a marked decrease in colonic dopamine D2 receptor expression, together with an increase in angiotensin AT1, a decrease in angiotensin AT2 receptor expression (i.e. an increase in the RAS pro-inflammatory arm activity) and increased levels of inflammatory and oxidative markers. Aged rats also showed increased levels of colonic dopamine and noradrenalin, and marked decrease in acetylcholine and serotonin levels. The present observations contribute to explain an aging-related pro-inflammatory state and dysregulation in gastrointestinal function, which may be counteracted by treatment of aged animals with the AT1 receptor blocker candesartan.

POSTER 19

Principal Investigator: Pérez Tur, Jordi (CIBERNED, Valencia).

Title: **Evaluation of a novel mutation in patients with Primary Lateral Sclerosis (PLS).**

Authors: Szymanski J, Rubio C, Cardona F, Vázquez-Costa JF, Pérez-Tur J.

Abstract: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder with a mean prevalence of 5.40/100.000 inhabitants in Europe. A clear cause of this fatal neurodegenerative disorder is unknown, thus an effective cure has not been developed. A novel mutation in a gene that cannot be disclosed at this time was found in family members affected by primary lateral sclerosis (PLS), a disease that is part of the spectrum of ALS. The gene in question encodes a protein involved in many essential cellular processes. Results were obtained using whole exome sequencing and confirmed using Sanger sequencing. Allele-specific PCR revealed the presence of this single nucleotide polymorphism (SNP) in members of the family affected by PLS but not the controls examined. An in silico study has shown a possible negative effect of the mutation on the active site of the protein. Site-directed mutagenesis was used to introduce the SNP into a plasmid expressing the protein. Both the wild type and mutant vectors were introduced into Escherichia coli DH5? strain. The subsequently extracted proteins were used in an activity assay to compare their efficiencies. Frequency, at which the SNP occurs only in the affected family, indicates possible significance. The involvement of the protein in essential cellular processes suggests it may be, or may interact with possible targets for therapy.

POSTER 20

Principal Investigator: Pérez Tur, Jordi (CIBERNED, Valencia).

Title: **Development of a PCR-dot-blot method to detect C9orf72 expansions.**

Authors: Esteller P, Vaca M, Villafranca M, Pérez-Tur J, Cardona F.

Abstract: Amyotrophic lateral sclerosis (ALS) is the most common type of muscular neurodegenerative disease with fatal prognosis and for which there is no cure available yet. It is a complex heterogeneous disease whose etiology remains unknown. Moreover, the genetic component only contributes to 5-10% of the cases while the remaining 90-95% of the cases are classified as sporadic. Often this disease is accompanied by Fronto-Temporal-Dementia (FTD). Several loci have been associated with the disease, among which C9orf72 has been recently identified; in which a non-coding GGGGCC hexanucleotide repeat expansion has been assumed to be the responsible for almost half of the genetic ALS-FTD cases. Moreover, expansions in such gene have also been associated, albeit less frequency, to other neurological diseases both motor and non-motor ones. C9orf72 expansion detection methods used in diagnosis combine repeat-primed PCR and fragment analysis along with Southern blot but have the limitation of being too costly and/or time consuming. The present projects aims to develop a fast detection method for such expansions based on dot blot techniques using specific DNA probes. Once such methodology has been developed, it could be applied for mass analysis in order to establish the prevalence of these expansions in other neurological diseases. It could also be used as a filtering method prior to performing the Southern blot technique. Key words: Amyotrophic lateral sclerosis, C9orf72, dot blot, Southern blot, DNA probes.

POSTER 21

Principal Investigator: Fernández Ruiz, Javier (CIBERNED, Madrid).

Title: **Evaluation of the neuroprotective potential of IGS-2.7, a casein kinase-1 inhibitor, in TDP-43 transgenic mice, a model of amyotrophic lateral sclerosis.**

Authors: Cabezudo D, Martínez-González L, Pérez DI, Fernández-Ruiz J, Martínez A, de Lago E.

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease affecting motor neurons, both upper and lower, which unfortunately has no cure and useful treatments to date. The only approved medicine is riluzole (Rilutek®), an antiglutamatergic agent but with very modest effects. Therefore, there is an unmeet need of new treatments concentrated not only in alleviating specific symptoms (e.g. cramps, fasciculations, spasticity), but useful to delay disease progression. With this objective in mind, we have conducted a preclinical investigation with the compound IGS-2.7, an inhibitor of casein kinase-1 (CK1), for its potential as neuroprotectant in a genetic model of ALS: TDP-43 transgenic mice. TDP-43 is a DNA-binding protein, which is involved in mRNA regulation and processing. Mutations in TDP-43 have been found to elevate levels of CK1 in affected neurons, which derives in TDP-43 phosphorylation. Such event contributes significantly in the onset and progression of ALS, so that inhibition of CK1 has been proposed as an interesting strategy in ALS as well as in other pathologies showing TDP-43 aggregation. In our study, we administered IGS-2.7 (1 mg/kg/day) to adult male Prp-hTDP43A315T transgenic and wild-type mice from the age of 65 days till to 90 days. The treatment with IGS-2.7 was able to attenuate the weight loss experienced by TDP-43 transgenic mice, although it had no effects on the deterioration in rotarod performance reflecting muscle weakness. However, the histopathological analysis of their spinal cords, using Nissl staining or immunostaining for choline-acetyl transferase (ChAT), showed a marked preservation of motor neurons in the ventral horn in TDP-43 transgenic mice treated with IGS-2.7. This was accompanied by a reduction in reactive gliosis labelled by GFAP immunostaining. In summary, CK1 inhibition may represent a useful therapeutic strategy for a neuroprotective treatment in ALS, at least in those cases having TDP-43 alterations. However, such strategy should be improved with more potent inhibitors or combinations with other active agents, with the purpose to get that the IGS-2.7-induced preservation of spinal motor neurons leads to a recovery in functional parameters.

POSTER 22

Principal Investigator: Fernández Ruiz, Javier (CIBERNED, Madrid).

Title: **Investigation in the anti-inflammatory and neuroprotective effects of VCE003.2, a cannabigerol quinone derivative, using inflammation-based cellular models and in vivo experimental parkinsonism.**

Authors: Burgaz S, Gómez-Cañas M, Bellido ML, Navarrete C, Muñoz E, García C, Fernández-Ruiz J.

Abstract: Phytocannabinoids and their derivatives have shown a promising potential for developing neuroprotective therapies against neurodegenerative conditions. In the present study, we investigated a quinone derivative of the non-psychotrophic phytocannabinoid cannabigerol (CBG), named VCE003.2, which, given its CBG structure, is a potent antioxidant with no activity at the cannabinoid receptors, but being able to activate PPAR-? receptors. We first confirmed the lack of affinity of VCE003.2 for the cannabinoid receptors using competition studies (Ki >40 µM), as well as its transcriptional activity as PPAR-? activator using a luciferase-based reporter assay. Next, we investigated VCE003.2 activity against lipopolysaccharide (LPS)-induced activation of microglial cells (using cultured BV2 cells). We found that LPS exacerbated the proinflammatory/neurotoxic profile of these cells (elevated synthesis and release of TNF-? and IL-1?, as well as induction of proinflammatory enzymes such as cyclooxygenase-2 and inducible nitric oxide synthase), whereas the pretreatment with VCE003.2 reversed these effects. Next, we wanted to investigate the issue in an in vivo inflammatory model of neuronal damage. We used mice subjected to unilateral intrastriatal injections of LPS, which is reminiscent of inflammatory events occurring in Parkinson's disease. We found that this CBG derivative was efficacious in recovering the loss of tyrosine hydroxylase (TH)-containing nigrostriatal neurons and, in particular, in attenuating the intense microgliosis provoked by LPS in the substantia nigra, measured by using TH and Iba-1/Cd68 immunostaining, respectively. The analysis by qPCR of proinflammatory cytokines (e.g. TNF-?, IL-1?) and enzymes (e.g. inducible nitric oxide synthase) in the substantia nigra showed they were markedly elevated by the LPS lesion and strongly reduced by the treatment with VCE003.2. Lastly, we observed that these beneficial effects of VCE003.2 in LPS-lesioned mice likely implied the activation of PPAR? receptors, as they disappeared when VCE003.2 was co-administered with the PPAR? inhibitor T0070907. In summary, we demonstrated that VCE003.2 is anti-inflammatory and neuroprotective in models of microglial activation and neuronal damage, and we proved that its efficacy, at least in conditions of marked inflammation, may be mediated by the activation of PPAR?. Supported by "Ministerio de Economía y Competitividad, Programa Retos-Colaboración" (RTC-2014-1877-1), "Programa Nacional de Biomedicina" (SAF2015-68580-C2-1-R) and VivaCell Biotechnology-Spain.

POSTER 23

Principal Investigator: Lucas Lozano, José Javier (CIBERNED, Madrid).

Title: **Translational regulation by RNA-binding proteins as a common mechanism for neurodevelopmental and neurodegenerative disorders.**

Authors: Parras A, Anta H, Santos-Galindo M, Elorza A, Picó S, Hernández IH, Wang N, Tomás-Zapico C, Fernández-Nogales M, Ferrer I, Yang XW, Navarro P, Méndez R, Lucas JJ.

Abstract: Translational control of specific mRNAs is a widespread mechanism of gene regulation that allows for rapid changes in concentrations of the encoded proteins in the entire cell or in a given subcellular compartment. mRNA silencing and localization combined with spatially restricted reactivation allow for rapid response to extracellular signals and stress thus resulting in spatiotemporal control of gene expression during development, differentiation and nervous system function. Not surprisingly, alterations in translational control have been associated with aging, and disease. Regulatory sequences in the 5' and 3' untranslated regions (UTRs) and their cognate RNA binding proteins provide this transcript specific regulation by recruiting cofactors that assemble translational-repression or -activation ribonucleoprotein particles (mRNPs). Here we find alteration of this kind of mRNPs in Huntington's disease and identify common targets involved in neurodevelopmental disorders which also correlate with aberrant regulation of translation (one important example is fragile X mental retardation syndrome). We conclude that translational regulation by RNA-binding proteins emerges as a common mechanism for neurodevelopmental and neurodegenerative disorders.

POSTER 24

Principal Investigator: Naranjo Orovio, José Ramón (CIBERNED, Madrid).

Title: **Role of DREAM-ATF6 interaction in Alzheimer's disease.**

Authors: Lopez-Hurtado A, González P, Dopazo XM, Cercós P, Gutérrez-Rodríguez M, Mellström B, Naranjo JR.

Abstract: A defective unfolded protein response (UPR) has been implicated in a variety of neurodegenerative disorders, including Alzheimer's disease (AD). In AD, formation of Aβ oligomers and multimers is associated with reduced ATF6 processing and with chronic activation of the PERK pathway, two hallmarks of deregulated UPR. While reduced ATF6 activity weakens the pro-survival phase of the UPR, activated PERK potentiates a pro-apoptotic response. Thus, defective UPR is an attractive potential target for therapy and PERK inhibitors are being explored though their used has been hampered by unwanted side effects.

Our team recently reported that the interaction between ATF6 and the neuronal calcium sensor DREAM regulates ATF6 processing, and inhibitors of this interaction have been effective delaying the onset and the progression of disease symptoms in mouse models of Huntington disease. In this study, we analyzed whether a parallel mechanism occurs in AD, and whether disruption of the DREAM-ATF6 interaction could also improve UPR in this pathology. For that, we assayed changes in DREAM protein levels and ATF6 processing in the hippocampus and the cerebral cortex from J20 mice, a mouse model of AD. The analysis was performed at different stages during pre- and symptomatic phases and the results in transgenic and wild type littermates compared.

Contrary to the results observed in the striatum from R6/2 mice, a mouse model of Huntington disease, hippocampal levels of transcriptionally active ATF6 were increased in J20 mice at all stages during the pre-symptomatic phase compared to wild type littermates. In symptomatic 12-months old J20 mice, like in R6/2 mice, levels of nuclear ATF6 were decreased and this reduction was prevented in mice chronically receiving repaglinide, a DREAM binding molecule able to disrupt the DREAM-ATF6 interaction.

These results suggest that the DREAM-ATF6 interaction may be also a target to normalize UPR in mouse models of AD.

Funded by grants from CIBERNED and MICINN (SAF2012-32209; SAF2014-53412-R).

POSTER 25

Principal Investigator: Naranjo Orovio, José Ramón (CIBERNED, Madrid).

Title: **The neuronal calcium sensor DREAM: a drugable target to modulate presenilin-2 activity in Alzheimer disease.**

Authors: Naranjo R, González P, Dopazo XM, Cercós P, Gutiérrez-Rodríguez M, Mellström B, Naranjo JR

Abstract: Deregulated intracellular Ca2+ and protein homeostasis underlie synaptic dysfunction and are common features in neurodegenerative diseases. DREAM/calsenilin/KChIP-3 is a multifunctional Ca2+ binding protein of the neuronal calcium sensor superfamily with specific functions in different subcellular compartments. In the endoplasmic reticulum (ER), we have recently disclosed that DREAM controls ATF6 processing and the pro-survival phase of the unfolded protein response (UPR) in the striatum of R6/2 mice, a mouse model of Huntington disease. Molecules, like repaglinide, able to bind DREAM and disrupt the DREAM-ATF6 interaction block this activity of DREAM and delay onset and progression of disease symptoms in HD mice. Also in the ER, it was shown that DREAM interacts with presenilins (PS-1 and PS-2) regulating their activity as part of the g-secretase complex in the processing of the amyloid precursor protein (APP), the cleavage of the presenilin C-terminal part and the release of calcium from the ER. Mutations in the presenilin genes are often associated with the development of familial type of Alzheimer disease (AD). Furthermore, genetic ablation of DREAM leads to reduced APP processing and lower levels of Ab peptides. In this study we have analyzed the ability of DREAM binding molecules, like repaglinide, to disrupt the DREAM-PS2 interaction and by hence to reduce the formation of the C-terminal PS-2 fragment. In vitro co-immunoprecipitation assays showed that DREAM binding molecules, in the nanomolar range, inhibit the DREAM-PS-2 interaction, while in vivo experiments revealed their ability to reduce the formation of the C-terminal PS-2 fragment in neuroblastoma cells. These results suggest that the DREAM-PS-2 interaction may also be a target to normalize PS-2 activity in AD.

POSTER 26

Principal Investigator: Ávila de Grado, Jesús (CIBERNED, Madrid).

Title: **Novel function of tau in regulating the effects of external stimuli on adult hippocampal neurogenesis.**

Authors: Pallas-Bazarra N, Jurado-Arjona J, Terreros-Roncal J, Navarrete M, Esteban JA, Hernández F, Ávila J, Llorens-Martín M.

Abstract: Tau is a microtubule-associated protein found mainly in axons. However, its presence in dendrites and dendritic spines has acquired special relevance due to its involvement in synaptic plasticity. One of the most drastic examples of plasticity in the brain is the addition of new neurons to a preexisting circuitry during the adulthood. Noteworthy, the addition of new neurons to the hippocampal circuit is regulated by numerous external factors, thus conferring an outstanding degree of plasticity to the network. Interestingly, alterations in adult hippocampal neurogenesis appear to be a relevant neuropathological feature of a group of neurodegenerative diseases known as tauopathies, characterized by impaired Tau metabolism. Thus, we aimed to investigate the role of Tau in the regulation of this process exerted by both detrimental (acute stress) and stimulatory (environmental enrichment, EE) external stimuli. By using a Tau knockout mice model (Dawson et al, 2001), we demonstrate that Tau plays a novel in vivo role in the morphological and synaptic maturation of newborn granule neurons under basal conditions. Moreover, our data reveal that Tau deficiency prevents the selective apoptosis of immature granule neurons caused by acute stress. What´s more, it protects newborn neurons from the stress-induced dendritic atrophy and loss of postsynaptic densities (PSDs). Strikingly, Tau also regulates the increase in newborn neuron survival triggered by EE. Furthermore, newborn granule neurons from Tau knockout mice do not show any stimulatory effect on dendritic development or on PSD generation. Thus, this work reveals a novel role of Tau in the maturation of newborn granule neurons in vivo under basal conditions. Furthermore, we provide evidence that Tau regulates the effects of external stimuli on adult hippocampal neurogenesis, since newborn granule neurons from Tau knockout mice are insensitive to the modulation exerted by both stimulatory and detrimental stimuli. PUBLICATION: Pallas-Bazarra N, Jurado-Arjona J, Navarrete M, Esteban JA, Hernandez F, Avila J, Llorens-Martin M (2016) Novel function of Tau in regulating the effects of external stimuli on adult hippocampal neurogenesis. The EMBO journal BIBLIOGRAPHY: Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP (2001) Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J Cell Sci 114: 1179-1187.

POSTER 27

Principal Investigator: de Felipe Oroquieta, Javier (CIBERNED, Madrid).

Title: **Senile plaques characterization in CA1 in patients with Alzheimer's disease.**

Authors: Furcila D, De Felipe J, Alonso-Nanclares L.

Abstract: Alzheimer's disease (AD) is the most common form of dementia which produce cognitive impairment and cerebral degeneration. Its main hallmarks are the presence of aggregates of β-amyloid peptide (Aβ) forming senile plaques, and hyperphosphorylated tau (PHF-Tau) inside neurons, so called neurofibrillary tangles (NFTs). Studies on the correlation of Aβ plaques and NFTs with cognitive impairment propose that despite Aβ plaques may play a key role in AD pathogenesis, the severity of cognitive impairment correlates best with the burden of PHF-Tau. Several studies have reported that CA1 is one of the most affected areas of the hippocampus due its direct connections with other cortical regions and the presence of PHF-Tau inside pyramidal neurons. The present study analyzes the senile plaques in CA1 in 11 AD patients (ages between 76 and 95 years in the decease moment). Entire CA1 region have been examined using double and triple immunofluorescence and confocal microscopy to examine the plaque distribution and the expression patterns of Aβ and PHF-Tau proteins in CA1 plaques. We found that most plaques are located in medial and distal regions of CA1 (respect the dentate gyrus), and co-localization analysis of double immunofluorescence (anti-Aβ/anti-PHF-Tau-PHF1, anti-Aβ/anti-PHF-Tau-AT8 and anti-PHF-Tau-PHF1/anti-PHF-Tau-AT8) shows a variety of overlapping patterns of Aβ and PHF-Tau proteins in CA1 plaques. Moreover, triple labelling (Methoxy-X04/anti-PHF-Tau-PHF1/anti-PHF-Tau-AT8) allowed the visualization of "tau plaques" which only displayed immunofluorescence for anti-PHF-Tau markers. This study provides a detailed examination of senile plaques in CA1 region suggesting that both Aβ and PHF-Tau proteins in senile plaques act together, as previously reported. Future research might consider to study the formation and growth of plaques with more antibodies (e.g. anti-PHF-Tau), and not only with anti-Aβ specific markers, as it can clarify and offer a better understanding of the interaction between both altered proteins in all stages of the disease.

POSTER 28

Principal Investigator: Matute Almau, Carlos (CIBERNED, Bilbao).

Title: **Astrocytes contribute to the spreading of pathogenic ?-synuclein.**

Authors: Cavaliere F, Ramos P, Dehay B, Bezard E, Obeso J, Matute C.

Abstract: Alpha synuclein (?-syn) is the main protein component of Lewy Body (LB) aggregates, one of the pathological hallmarks of Parkinson´s Disease. This protein can spread through the brain in a "prion-like" manner. Understanding the mechanism of cellular processing and spreading of ?-syn is essential to understand the progression of PD. In this work we investigated the contribution of astrocytes to the spreading of pathogenic ?-syn and the onset of neuronal death. First, we treated rat cells (neurons and astrocytes) with human LB extracts obtained from post-mortem brains of PD patients to study internalization of exogenous ?-syn (h?-syn) contained in the human LB. We found that h?-syn is taken up by neurons and astrocytes by endocytosis and localizes at subcellular sites different from the endogenous ?-syn. Moreover, we observed by microfluidic assay that h?-syn can be intracellularly transported in all cell types and metabolized by lysosomes to a major extent in astrocytes than in neurons. Finally, we found that internalization of exogenous h?-syn triggers an increase of the endogenous ?-syn levels, which is more significant in neurons than in astrocytes making the former more sensitive to apoptosis. Together, these results indicate that astrocytes contribute to the spreading of toxic ?-syn and that targeting astroglia may help halting PD progression.
Supported by CIBERNED and CoEN. PR is the recipient from a fellowship from Gobierno Vasco.

POSTER 29

Principal Investigator: Matute Almau, Carlos (CIBERNED, Bilbao).

Title: **White matter pathology in Alzheimer´s disease: Role of amyloid beta oligomers in oligodendrocyte dysfunction.**

Authors: Quintela-López T, Wyssenbach A, Ortiz-Sanz C, Pérez-Samartín A, Möbius W, Ruhwedel T, Nave KA, Matute C, Alberdi E.

Abstract: Amyloid pathology significantly affects white matter, a feature which may be relevant to Alzheimer´s disease (AD). Knowledge about AD-associated alterations in oligodendrocyte and myelin is scant and it is commonly regarded as secondary to the disease itself or to aging. Here, we report that oligomeric amyloid ? peptide (A?) interferes directly with oligodendrocytes. First, we characterized the effects of oligomeric amyloid ? peptide (A?) in primary oligodendrocytes function. We observed that A? oligomers regulate local translation of myelin basic protein (MBP), promoting an increase of this protein levels. Activation of ?1-integrin receptor, Fyn and CREB proteins were involved in the molecular mechanisms underlying A?-induced MBP increase. We also examined whether A? accumulation in vivo was associated with MBP dysregulation. Western blotting of MBP and A? oligomers in hippocampal formation and corpus callosum of 18-month-old triple-transgenic mouse model of AD (3xTg-AD) showed an increase of MBP levels which correlated with A? oligomer burden. In addition, 3xTg-AD mice exhibited a higher number of mature and progenitor oligodendrocyte cells (CC1+ and PDGFR-?+cells, respectively) and nodes of Ranvier than matched controls. Ultrastructural analysis of corpus callosum samples of 3xTg-AD mouse revealed several myelin degenerated events, having thicker myelin sheath (i.e., lower g-ratio) and an enlarged inner tongue. No differences were found in both, caliber and number of myelinated axons between 3xTg-AD and wild type mice. To determine the functional consequences of these white matter defects, we measured compound action potentials in corpus callosum of 18-month-old transgenic mice. The conduction velocity was significantly lower in myelinated, but not in unmyelinated axons of transgenic mice compared to wild type mice. Consistent with these findings, higher MBP levels were detected in the human hippocampal formation, specifically in CA3 and dentate gyrus, and frontal cortex in advanced stages of AD. Additionally, western blot analysis of myelin proteins, MBP and CNPase, in cerebrospinal fluid of subjects with different level of cognitive impairment, revealed elevated protein levels in mild cognitive impairment comparing to subjective cognitive impairment patients. In conclusion, these results reveal the presence of a myelin sheath pathophysiology in AD which may lead to altered neuronal communication, being unclear the role of the MBP upregulation. Funded by CIBERNED, MINECO and Gobierno Vasco. TQ-L is a recipient of a fellowship from the Gobierno Vasco and CO-S from UPV/EHU.

POSTER 30

Principal Investigator: Rodríguez Álvarez, José (CIBERNED, Barcelona).

Title: **Analysis of synaptic-related microRNAs expression in Alzheimer's disease.**

Authors: Siedlecki-Wullich DJ, Català-Solsona J, Fábregas-Ordóñez C, Aguilera J, Saura CA, Rodríguez-Álvarez J, Miñano-Molina AJ.

Abstract: MicroRNAs (miRNAs) are small non-coding RNA molecules that fine-tune gene expression at post-transcriptional level. Recent studies have shown that deregulation of specific miRNAs could be involved in the development and progression of Alzheimer's disease (AD). However, few studies have explored the relationship between miRNAs deregulation in AD and synaptic plasticity despite the involvement of some miRNAs in synaptic plasticity. Moreover, a comprehensive analysis of the miRNome during AD progression is lacking. In this study, we performed a microarray analysis (Affymetrix® miRNA 4.1) in the entorhinal cortex, hippocampus, prefrontal cortex and cerebellum of AD patients at different Braak stages. We found that 47 miRNAs were deregulated in the entorhinal cortex (17 down-regulated and 30 up-regulated) and 23 in the hippocampus (17 down-regulated and 6 up-regulated) at Braak stage I/II compared to age-matched non-demented controls. miRNAs targeting synaptic-related mRNAs were identified using the miRWalk database and validated by RTqPCR. qPCR analysis confirms a reduction of several miRNAs including miR-92a-3p (log2 relative expression = -0,7 ± 0,33), miR-181c-5p (log2 relative expression = -0,46 ± 0,33) and miR-210-3p (log2 relative expression = -1,16 ± 0,42) levels during early stages of AD (Braak I/II) in the entorhinal cortex, but not in the hippocampus. Our results show altered levels of specific miRNAs, related to synaptic activity regulation, during early AD pathology. Support: This work was supported by grants from Ministerio de Economia y Competitividad (SAF2014-59697-R), CIBERNED (CB06/05/0042), Fundació Marato TV3 (2014- 3610) and Generalitat de Catalunya (SGR2009-1231).

POSTER 31

Principal Investigator: Trullás Oliva, Ramón (CIBERNED, Barcelona).

Title: **Mitochondrial DNA in cerebrospinal fluid distinguishes idiopathic from LRRK2-related Parkinson's disease.**

Authors: Podlesniy P, Vilas D, Taylor P, Shaw LM, Tolosa E, Trullás R.

Abstract: Mitochondrial DNA (mtDNA) regulates mitochondrial function and encodes genes that are essential to provide the energy that neurons require to survive and sustain their activity. Mitochondrial dysfunction is associated with both idiopathic and familial forms of Parkinson's disease. To investigate whether these two disease forms exhibit an altered mtDNA regulation we measured cell free mtDNA content in cerebrospinal fluid (CSF) from idiopathic and LRRK2-related Parkinson's disease patients. The concentration of mtDNA was measured using a digital droplet polymerase chain reaction technique in a total of 98 CSF samples from a cohort of subjects including: 20 LRRK2-G2019S mutation carriers with Parkinson's disease, 26 asymptomatic LRRK2-G2019S mutation carriers, 31 patients with idiopathic Parkinson's disease and 21 first-degree relatives of LRRK2 Parkinson's disease patients without the mutation. We found that LRRK2-G2019S mutation carriers with Parkinson's disease exhibit a high concentration of mitochondrial DNA in CSF compared with asymptomatic LRRK2-G2019S mutation carriers and with idiopathic Parkinson's disease patients. In addition, idiopathic, but not LRRK2 Parkinson's disease is associated with low CSF concentration of alpha-synuclein. These results show that high mitochondrial DNA content in CSF distinguishes idiopathic from LRRK2-related Parkinson's disease suggesting that different biochemical pathways underlie neurodegeneration in these two disorders. Supported by Michael J Fox Foundation, MINECO, SAF2014-56644-R and by CIBERNED, PI2013/08-3.

POSTER 32

Principal Investigator: Wandosell Jurado, Francisco (CIBERNED, Madrid).

Title: **The effect of DHA diet on APP/PS1 females depends on the hormonal status.**

Authors: Herrera JL, Ordóñez-Gutiérrez L, Fàbrias G, Casas J, Salido E, Morales A, Hernández G, García-Segura LM, Alonso R, Wandosell F.

Abstract: Amyloid-? (A?) plaques in the brain constitute one of the hallmarks in Alzheimer's Disease (AD). Estradiol had been reported to reduce A? formation in murine primary cultures and in human cortical neurons, and to prevent from amyloid-? induced toxicity in cell lines. Additionally, high docosahexanoid acid (DHA) diets have been proven to diminish total A? and to prevent senile plaque formation in hippocampus and in parietal cortex of an amyloidosis mouse model. We have studied the synergistic effect caused by estrogens and DHA dietary supplementation in reducing A? levels in APP/PS1 transgenic females.

POSTER 33

Principal Investigator: Comella Carnice, Joan Xavier (CIBERNED, Barcelona).

Title: **Fas apoptosis inhibitory molecule (FAIM) knockout mice exhibits susceptibility to seizures.**

Authors: Calleja-Yagüe I, Coccia E, López-Soriano J, Gruart A, Jian-Xin H, Pérez-García MJ, Giménez-LLort L, Comella JX.

Abstract: Fas apoptosis inhibitory molecule (FAIM) gene encodes two alternative splicing forms, FAIM FAIM-S and FAIM-L. FAIM-S is ubiquitously expressed whereas FAIM-L is restricted to nervous system. In neurons, FAIM-S promoted NGF-induced neurite outgrowth through NF-?B and ERK signaling. FAIM-L, with additional 22 amino acids, protects from TNF?- or Fas-induced apoptosis by binding to Fas receptor preventing the activation of caspase 8, and by interaction and stabilization of XIAP. Previous results of our lab, show that FAIM-L can exert important functions in the central nervous system, related with synaptic transmission and plasticity (e.g. Long Term Depression). On the other hand, recent studies have implicated FAIM-L as a protective molecule in neurodegenerative disorders, particularly in Alzheimer´s Disease (AD). FAIM-L levels are decreased in the hippocampus of AD-mouse models and in post-mortem human tissue samples. Studies performed in the laboratory of DrHuo in FAIM knockout mice of both isoforms (FAIM-L and FAIM-S) showed that B-, T- cells and hepatocytes have increased sensitivity to Fas-triggered apoptosis. At the physiological level, the knock outs show spontaneous non-hyperphagic obesity. To date, the neuronal and behavioral phenotype of this model has not been described. Surprisingly, preliminary results in our lab show that FAIM knockout mice exhibit a noticeable susceptibility to convulsant seizures. Thus, the animals seem to be prone to suffer of recurrent seizure activity, which is characterized by generalized clonic seizures that are age-dependent and sensitive to manipulation (induced when handled). Interestingly, fast recovery times and a decrease of incidence after reexposure to stimuli point out to underlying mechanisms able to hamper such seizure activity. We are currently characterizing the behavioral and functional phenotype from young-adulthood to middle-age in order to assess their sensorimotor, cognitive and emotional profiles with the aim to find thresholds in their hyperexcitability patterns that may be refered to neuroanatomical and neurochemical profiles related to FAIM loss of function.

POSTER 34

Principal Investigator: Gutiérrez Pérez, Antonia (CIBERNED, Málaga).

Title: **Neurodegenerative process affects SOM and PV interneurons in Alzheimer's disease.**

Authors: Sánchez-Mejías E, Navarro V, Núñez-Díaz C, Jiménez S, Sánchez-Varo R, M Sánchez-Mico, Vizuete M, Dávila JC, Vitorica J, Gutiérrez A.

Abstract: Neuron loss is the best anatomopathological substrate that correlates with dementia in Alzheimer's disease (AD). According to the amyloid cascade hypothesis, specific neuronal networks are preferentially affected by amyloid-beta (A?) pathology during disease progression. Among these, inhibitory GABAergic interneurons have been shown to be highly vulnerable. In this sense, we have previously reported a substantial loss of somatostatin (SOM) interneurons in the hippocampus of APP/PS1 transgenic model, along with amyloid pathology progression, whereas parvalbumin (PV) subpopulation showed no change. Here, we analyzed the expression of SOM and PV, as markers of the major inhibitory populations, in the hippocampus and perirhinal cortex of post-mortem human AD brains in order to compare whit that observed in mice. For this purpose, RT-PCR, western blots and immunostainings were performed in mild (Braak II) to severe (Braak V-VI) AD cases and APP751SL/PS1M146L mice. In vitro studies to check the toxic effects of soluble S1 fractions on these GABAergic populations were also assayed. Results showed a significant decrease of SOM interneurons of Braak-VI in the dentate gyrus and the perirhinal cortex, along with a general reduction in SOM axonal plexus in all regions studied. Moreover, AD patients, unlike APP/PS1 model, displayed a significant reduction of the interneurons expressing PV. On the other hand, in the perirhinal cortex of APP/PS1 model there was a significant loss of SOM-positive cells in parallel to the extracellular accumulation of A?, however the neuronal population expressing PV remained stable during the course of the disease. In vitro studies also demonstrated the resistance of the PV-positive neurons to toxic agents (primarily A?) present in the soluble S1 fraction of transgenic models. Neurodegeneration of the interneurons might result in a loss of inhibitory innervation receiving principal neurons, leading to an imbalance of inhibition/excitation ratio and in consequence generating cognitive failures in learning and memory processes. The different vulnerability of PV-neurons in mice and patients, could be explained by the accumulation of soluble forms of phospho-tau and/or glia homeostasis dysfunction in AD patients. Supported by FIS-PI15/00796 (to AG) and FIS-PI15/00957 (to JV) co-financed by FEDER funds from European Union, and CTS-2035 from Junta Andalucia.

POSTER 35

Principal Investigator: Gutiérrez Pérez, Antonia (CIBERNED, Málaga).

Title: **Immunosuppression accelerates neuronal impairment in an APP/PS1 model of Alzheimer's disease.**

Authors: Sánchez-Varo R, Gómez-Gutiérrez R, Jiménez S, Navarro V, Baglietto-Vargas D, Vizuete M, Dávila JC, Vitorica J, Gutiérrez A.

Abstract: Previous works have demonstrated that neuroinflammatory response plays a key role in Alzheimer's disease (AD) progression. Microglia, the main cell type of the innate immune system in the brain, exhibit activation in both patients and animal models. However, and in contrary to animal models, we have recently reported a degenerative process of the microglial population in the hippocampus of AD patients (Braak V-VI). Therefore, an inefficient immunologic response could trigger an increase of amyloid-beta levels with disease progression. We aimed to investigate the effect of immunosuppression over the pathological progression in the hippocampus of APP/PS1dE9 transgenic mouse model. Cyclosporine (15 mg/kg) and prednisone (20 mg/kg) were intraperitoneally administered to these transgenic mice from 9 to 12 months of age. Untreated age-matched APP//PS1 mice were used as controls. Glial cell populations (microglia and astroglia), amyloid burden and neuritic pathology in the hippocampus were studied by immunohistochemistry, and data quantified by image analysis. Furthermore, cytokine production, glial activation, phospho-tau and A? levels, together with GABAergic neurodegeneration were evaluated using molecular techniques (qPCR and Western blots). The microglial marker Iba1 and some proinflammatory cytokines were found to be decreased by RT-PCR in the immunosuppressed mice compared to controls. In agreement with an immunosupressive status, reduced microglia activation around amyloid plaques was found in treated mice. Conversely, a significant increase in Abeta levels and the astroglial marker GFAP was detected. Moreover, the treatment negatively affected SOM and NPY subpopulations. Immunosuppression treatment downregulated microglial population activity and accelerated not only amyloid pathology but also the neuronal degeneration in this APP/PS1 model. Age-related deficiencies in the innate immune system could promote AD pathology and cognitive decline in patients. Therefore, regulating microglial activation signalling pathways might be considered as a therapeutical target for AD. Supported by FIS-PI15/00796 (AG), PI15/00957 (JV) co-financed by FEDER funds from European Union, PPIT.UMA.B1.2017/26 (RSV) and CTS-2035 from Junta de Andalucía.

POSTER 36

Principal Investigator: Carro Diaz, Eva (CIBERNED, Madrid).

Title: **Annexin V inhibits beta-amyloid-induced cytotoxicity in choroid plexus: implications for Alzheimer´s disease.**

Authors: Bartolomé F, Krzyzanowska A, Pascual C, Antequera D, García-Consuegra I, Villarejo A, Rábano A, Abramov AY, Ferrer I, Carro E.

Abstract: Annexin V (AV) has been shown to prevent amyloid-beta toxicity in neuronal cell cultures. Our previous study indicated enhanced AV expression in choroid plexus in very early stages (pre-clinical stages) of Alzheimer´s disease (AD) suggesting a putative protective role. OBJECTIVE: In this work the ability of AV to reduce cytotoxicity induced by amyloid-beta in choroid plexus will be investigated. METHODS: We used choroid plexus tissue samples from early stages of AD patients and primary choroid plexus cell cultures from rats. RESULTS: We observed that choroid plexus tissue samples from early stages of AD patients simultaneously exhibited enhanced AV levels and low amyloid-beta accumulation and cell death. In the primary choroid plexus cultures we observed that AV increased the cell viability in presence of amyloid-beta and inhibited the amyloid-beta toxicity. This process could also be linked to the mitochondrial damage as AV prevented the amyloid-beta-induced mitochondrial depolarisation as well as the subsequent mitochondrial calcium overload. Additionally, we also found AV modulated the autophagic events induced by amyloid-beta in the choroid plexus primary cultures. CONCLUSIONS: These results suggest that AV prevents amyloid-beta-induced citotoxicity in the choroid plexus modulating autophagy in a mitochondrial-dependent manner.

POSTER 37

Principal Investigator: Carro Diaz, Eva (CIBERNED, Madrid).

Title: **Analysis of the Expression of Taste and Olfactory Receptors in Choroid Plexus and Orbitofrontal Cortex of Alzheimer's Disease Patients.**

Authors: Cunha-Alves V, Figueiro-Silva J, Gonçalves I, Santos C, Ferrer I, Carro E.

Abstract: Background: Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized by progressive memory loss and cognitive deterioration, attributed to neuropathological lesions within specific regions in the brain. However, other areas of the brain such as the choroid plexus and the orbitofrontal cortex, have gained much attention since the choroid plexus is a multifunctional tissue responsible for a wide range of functions crucial to the central nervous system and the orbitofrontal cortex is considered among the most polymodal regions of the brain. Previous studies have shown the expression of taste and olfactory transduction pathways in rat choroid plexus as well as taste and olfactory regulation and activation of orbitofrontal cortex. Methods: Transcriptomic analysis of these chemoreceptors were performed by means of Real-time quantitative Polymerase Chain Reaction (RT-qPCR), and compared between AD patients (at different Braak stages of the disease) and age-matched controls. Results: Transcriptomic analysis indicated that orbitofrontal cortical olfactory receptors (ORs) and taste receptors (TASRs) are expressed and regulated at different stages of AD in male and female patients. Moreover, these receptors were differentially regulated at Braak stages I, V, VI, compared to age-matched controls in the orbitofrontal cortex of AD patients. The strongest differences were found at Braak stage I of AD. These receptors we also found to be expressed in choroid plexus, however, their expression is very low. Conclusions: Taken together, these results suggest that dysregulation of ORs and TASRs is an early event in the pathogenesis of AD. Nevertheless further studies are needed in order to strengthen these findings and to elucidate their potential physiologic functions in this brain area.

POSTER 38

Principal Investigator: Ferrer Abizanda, Isidro (CIBERNED, Barcelona).

Title: **Sativex in MCI patients at high risk of developing AD or at early stages of AD: a phase II clinical trial.**

Authors: Aso E, del Ser T, Cornet ME, Calero M, Strange B, Torres M, Medina M, Ferrer I.

Abstract: During the last few years, targeting the endogenous cannabinoid system has emerged as a potential therapeutic approach to treat Alzheimer's Disease (AD). Cannabinoids may target in parallel several processes that play key roles in this neurodegenerative disease, including Aβ and tau aberrant processing, chronic inflammatory responses, excitotoxicity, mitochondrial dysfunction and oxidative stress, among others. Interestingly, the administration of a combination of two compounds derived from the Cannabis sativa plant, which are the components of the already approved cannabis-based medicine Sativex®, resulted in cognitive improvement together with reduction of several pathologic parameters in APP/PS1 transgenic mice, a murine model of AD, both at early symptomatic and at more advanced stages of the disease progression. These findings have prompted the progression towards an exploratory, phase II, double-blind, four arm, placebo-controlled, randomized trial (Sat-CIEN-02), which has been already approved by the Spanish regulatory authorities and will be run in 9 hospitals of Madrid, Barcelona, San Sebastian and Santander. Sixty male and female patients with early dementia or mild cognitive impairment of Alzheimer type (Mini Mental State Examination (MMSE) >20; amyloid b42 < 500 pg/mL or tau > 450 pg/mL, or p-tau >50 pg/mL in CSF) will be administered by oromucosal route 1, 2 or 3 daily sprays of Sativex® or placebo for 26 weeks. The Primary Objectives are to evaluate the safety and tolerability of Sativex® and the changes in the cytokine levels in CSF as inflammatory markers. The effects of Sativex® on cognition (MMSE, ADAS-cog, Word fluency, Symbol-digit, Logical memory, Trial making test), behaviour (Neuropsychiatric Inventory), Global Clinical Assessment and CSF biomarkers will be examined as Secondary Objectives. The results are expected by the end of 2018. This phase II study will provide valuable safety and exploratory information about treatment of AD patients with cannabinoids.

POSTER 39

Principal Investigator: Lleó Bisa, Alberto (CIBERNED, Barcelona).

Title: **Network analysis of the CSF proteome identifies synaptic proteins as putative biomarkers for AD-related synapse loss.**

Authors: Belbin O, Núñez-Llaves R, Gómez-Giro G, Colom-Cadena M, Túnez-Bosch L, Balateu-Paños D, Muñoz-Llahuna L, Alcolea D, Morenas-Rodríguez E, Illán-Gala I, Fortea J, Bayés A, Lleó A.

Abstract: A biomarker capable of detecting synapse loss, which occurs early in Alzheimer's disease (AD) pathophysiology, would greatly assist in preclinical diagnosis, when treatment would most likely be effective. The objective of this study was to characterise the cerebrospinal fluid (CSF) proteome in terms of regional brain expression, functional and physical relationship to the synapse and interaction complexes in order to identify putative CSF biomarkers that could be used in the clinic to monitor the synaptic loss associated with Alzheimer's disease. Methods: A database of the CSF proteome was constructed by performing non-targeted liquid chromatography mass spectrometry on CSF samples from 50 cognitively healthy controls and 10 AD patients. A database of the synaptic proteome was constructed by literature curation of proteins detected in synaptosome-enriched fractions or annotated with a synaptic function in online databases. Results: Cross-referencing the CSF (2,731 proteins) and synaptic (537 proteins) proteomes produced a list of 165 proteins. This suggests that approximately 31% of the synaptic proteome is detectable in the CSF by shotgun mass spectrometry and 6% of the CSF proteome is of synaptic origin. Construction of an interaction map of the 165 proteins identified functional clusters of proteins that were used to select a panel of proteins for validation as CSF biomarkers for AD. Conclusions: By performing a detailed characterization of the CSF and synaptic proteomes and by confirming synapse expression in human brain, we have identified a panel of synaptic proteins detectable in the CSF which, if confirmed as biomarkers of synaptic loss, could be invaluable stage biomarkers for AD.

POSTER 40

Principal Investigator: Pastor Muñoz, María Asunción (CIBERNED, Pamplona).

Title: **Pooled-DNA sequencing reveals novel phenotypic associations in Parkinson's disease.**

Authors: Ortega-Cubero S, Benítez BA, Diez-Fairen M, Lorenzo-Betancor O, Cruchaga C, Lorenzo E, Samaranch L, Obeso JA, Rodríguez-Oroz MC, Aguilar M, Coria F, Pastor MA, Pastor P.

Abstract: Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra (Spain). 2Department of Neurology, Complejo Asistencial de Palencia (Spain). 3CIBER on Neurodegenerative Diseases (CIBERNED), Institute of Health Carlos III (Spain). 4Department of Internal Medicine, Washington University, St.Louis (USA). 5Fundació Docència i Recerca MútuaTerrassa (Spain). 6Memory and Movement Disorders Unit, Department of Neurology, Hospital Universitari MútuaTerrassa (Spain). 7Department of Neuroscience, Mayo Clinic, Jacksonville (USA). 8Department of Psychiatry and Hope Center Program on Protein Aggregation and Neurodegeneration, Washington University, St.Louis (USA). 9Centre for Integrative Neuroscience AC (CINAC), Hospital FM Puerta del Sur (Spain). 10CEU San Pablo University (Spain). 11Department of Neurology, Hospital Universitario Donostia; Neuroscience Unit, BioDonostia Research Institute (Spain). 12Clinic for Nervous Disorders, Hospital Universitari Son Espases (Spain). 13Department of Neurology; Neuroimaging Laboratory, Division of Neurosciences, CIMA, University of Navarra, Pamplona (Spain). Eighteen PARK loci and several susceptibility genes have been related to Parkinson's disease (PD). However, most studies focus on single genes in small PD series. Our aim was to establish the genetic background of a large Spanish PD sample. Pooled-DNA target sequencing of 7 major PD genes (SNCA, PARK2, PINK1, DJ-1, LRRK2, GBA, and MAPT) was performed in 562 PD cases (525 late-onset and 37 early-onset). Forty-four variants were found among 114 individuals (20.28%, $p<0.05$). Among these variants, thirty were found in Mendelian genes (68.18%) and 14 in PD susceptibility genes (31.82%). Seven novel mutations in heterozygous state were identified. Interestingly, most variants were found in PARK2 and PINK1 genes, whereas SNCA and DJ-1 alterations were rare. Heterozygous PARK2 variant carriers presented earlier disease onset age and showed dementia more frequently. PD subjects carrying two variants at different genes (1.31%) had an earlier onset age and a predominantly akinetic-rigid PD phenotype (55.6%, $p<0.05$), suggesting that the accumulation of genetic risk variants could modify PD phenotype.

POSTER 41

Principal Investigator: Cantero Lorente, José Luis (CIBERNED, Sevilla).

Title: **APOE4 hinders successful compensation of hippocampal atrophy in mild cognitive impairment.**

Authors: Prieto-del Val L, Cantero JL, Baena D, Atienza M.

Abstract: In mild cognitive impairment (MCI), APOE4 is associated with accelerated memory decline, likely because brain deterioration hampers successful compensation. To test this hypothesis, we examined human hippocampal volume and neocortical thickness using MRI, and estimated cortical sources of EEG oscillations during memory retrieval. Twenty-six healthy older adults and thirty-four individuals with amnestic MCI, of which sixteen were APOE4 carriers, participated in the study. All patients showed hippocampal volume reduction and cortical thinning and dysfunctional EEG oscillations across fronto-temporal areas. But importantly, APOE4 status was the critical factor in determining the impact of temporal degeneration on memory. Specifically, path analyses revealed that right hippocampal atrophy in MCI was directly responsible for memory deterioration in APOE4 carriers, a causal relationship mediated by the serial intervention of three related factors in noncarriers. Temporal cortical thickness (first mediator) accounted for activation of functional compensatory networks through synchronized theta activity across temporal regions (second mediator), which, in turn, coordinated memory reactivation through desynchronized alpha/beta activity across sensorimotor areas (third mediator). Results revealed that, contrary to APOE4-carrier patients, noncarriers are successful in compensating for memory decline as long as the integrity and functionality of the temporal lobe is preserved, a fact primarily dependent on hippocampal degeneration.

POSTER 42

Principal Investigator: Cantero Lorente, José Luis (CIBERNED, Sevilla).

Title: **Sleep quality mediates the association between homocysteine and oxidative status in mild cognitive impairment.**

Authors: Sánchez-Espinosa MP, Atienza M, Cantero JL.

Abstract: Tremendous progress has been made over the last few years in understanding how sleep and amyloid-? (A?) cooperate to speed up the progression of Alzheimer's disease (AD). However, it remains unknown whether sleep deficits also interact with other risk factors that exacerbate the pathological cascade of AD. Based on evidence showing that higher levels of homocysteine (HCY) and sleep loss increase oxidative damage, we here investigate whether the relationship between HCY and total antioxidant capacity (TAC) is mediated by changes in objective sleep in healthy older (HO, N=21) and mild cognitive impairment (MCI, N=21) subjects. Results revealed that reduced TAC levels in MCI was significantly correlated with increased HCY, shorter sleep duration, lower sleep efficiency, and reduced volume of temporal regions. However, only the HCY-TAC association showed diagnostic value, and this relationship was mediated by poorer sleep quality in MCI patients. We further showed that HCY-related cerebral volume loss in MCI depended on the serial relationship between poorer sleep quality and lower TAC levels. These findings provide novel insights into how impaired sleep may contribute to maintain the relationship between HCY and oxidative stress in prodromal AD, and offer empirical foundations to design therapeutic interventions aimed to weaken this link.

POSTER 43

Principal Investigator: Fernández Chacón, Rafael (CIBERNED, Sevilla).

Title: **Functional genetics of Cysteine String Protein-alpha in the maintenance of skeletal muscle fibers and satellite stem cells.**

Authors: López-Begines S, Arroyo-Saborido A, Muñoz-Cánoves P, Fernández-Chacón R.

Abstract: The functional decline of the nervous and the musculoskeletal systems is a major factor determining the quality of life in elderly populations. Nowadays, it is well established that stem cells in specific neural and muscular niches, active throughout life, are essential for the functional maintenance of the brain and skeletal muscles. Stem cell properties, though, are sensitive to aging and to pathogenic factors such as molecular insults underlying neurodegeneration. Nevertheless, the molecular mechanisms that progressively hinder stem cells regenerative potential are poorly understood. CSP-alpha is a vesicle-associated molecular co-chaperone that contains a Dnaj domain. Nerve terminals require CSP-alpha to maintain their integrity. In humans, CSP-alpha mutations cause autosomal-dominant neuronal ceroid lipofuscinosis, a neurodegenerative disorder associated to dementia and early death. We have recently found that CSP-alpha is essential to maintain postnatal quiescence in neural stem cells (Gomez-Sánchez, Nieto-González et al. unpublished). Interestingly, according to microarray data (Sousa-Victor et al. Nature, 2014), CSP-alpha is present in muscular satellite stem cells. However, the function of CSP-alpha in satellite cells is unknown. We have used genetically modified mouse stem cells bearing a DNAJC5 floxed allele (European Conditional Mouse Mutagenesis Program, Skarnes et al. Nature 2011) to generate CSP-alpha conditional knock-out mice. Next, we have bred those mice against transgenic mice expressing Cre recombinase and a fluorescent marker (YFP) specifically in satellite cells under the Pax-7 promoter. We expect our in vivo approach will shed light on the function of CSP-alpha in satellite cells and in the long term maintenance of skeletal muscular fibers.

POSTER 44

Principal Investigator: Navarro Acebes, Xavier (CIBERNED, Barcelona).

Title: **Effects of Interleukin-37 in Amyotrophic Lateral Sclerosis.**

Authors: Martínez-Muriana A, Dinarello CA, López-Vales R.

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that affects upper and lower motor neurons (MNs). MNs loss results in skeletal muscle weakness, spasticity and paralysis, leading to the death of patients by respiratory failure 3 to 5 years after diagnosis. As occurs in several neurological disorders including ALS, inflammation is a pathological hallmark. Several reports have provided evidence that glial cells and leukocytes accelerate the course of the disease in animal models of ALS, suggesting that therapies aimed at targeting inflammation may be a valuable approach for this neurodegenerative disease. IL-37 is the only member of the IL-1 family that exerts broad anti-inflammatory effects over innate and acquired immunity. We have previously demonstrated that IL-37 mediates marked anti-inflammatory actions in the injured central nervous system, and confers protection against secondary tissue damage and functional deficits after spinal cord contusion injury in mice. However, whether IL-37 exerts similar beneficial effects in neurodegenerative conditions is not known yet. In the present study, we investigated whether IL-37 suppresses inflammation and slows the clinical course of the disease in a mouse model of ALS (SOD1G93A mouse). Since no mouse genomic sequence corresponding to human IL-37 has been found yet, we crossed transgenic mice expressing the human form of IL-37 with SOD1G93A mice to assess the effects of IL-37 in ALS. Our data reveals that transgenic expression of IL-37 slowed ALS disease progression, extended lifespan of SOD1G93A mice, and increased the preservation of spinal cord motoneurons. We also observed that IL-37 attenuated microgliosis and astrogliosis in the lumbar spinal cord of SOD1G93A mice. Since microgliosis and astrogliosis are regulated, in part, by cytokines, we assessed whether IL-37 modulated the protein levels of several cytokines in the sciatic nerve and spinal cord of ALS mice. Luminex experiments revealed that IL-37 attenuated the expression of IL-4 and IL-6 in the sciatic nerve of SOD1G93A mice and reduced the levels of MCP1 in both, sciatic nerve and spinal cord. Interestingly, IL-37 increased in 4 fold the protein levels of the anti-inflammatory cytokine IL-10 in the spinal cord. This data demonstrates the anti-inflammatory and beneficial actions of IL-37 in a mouse model of ALS.

POSTER 45

Principal Investigator: Navarro Acebes, Xavier (CIBERNED, Barcelona).

Title: **Neuregulin 1 reduces motoneuron cell death, neuroinflammation and promotes neurite outgrowth in an Amyotrophic Lateral Sclerosis in vitro model.**

Authors: Mòdol-Caballero G, Herrando-Grabulosa M, Santos D, Navarro X.

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder with no cure currently available. The pathogenic processes of the disease are likely to be multifactorial; a complex interplay between multiple pathogenic mechanisms causes motoneuron degeneration. Although the motoneuron death mechanism is still unclear, glutamate excitotoxicity and neuroinflammatory reaction are two main features in the neurodegenerative process of ALS. Recently it has been shown that Neuregulin 1 (NRG1), a trophic factor highly expressed in motoneurons and neuromuscular junctions, was found reduced in patients with ALS. We used an in vitro model based on the chronic excitotoxic response caused by the compound DL-threo-?-Hydroxyaspartic acid (THA) to characterize the effect of NRG1 on the motoneuron survival. For this purpose we applied the recombinant human NRG1 (rhNRG1) to the spinal cord organotypic cultures. Our results show that rhNRG1 treatment is able to preserve the motoneurons and to reduce the microglial reactivity overcoming the excitotoxic effects caused by THA. Furthermore rhNRG1 activates the PI3K/AKT pathway through the ErbB receptors and reduces the expression of the LC3-II autophagosome marker. Moreover, rhNRG1 promotes motor and sensory neurite outgrowth. In conclusion, exogenous rhNRG1 promotes motoneuron preservation by activating the ErbB2/3/4 receptors. This activation leads to a reduction of microglial reactivity and an increase of motoneuron survival through the AKT pathway, and regulation of autophagy.

POSTER 46

Principal Investigator: López de Munain Arregui, Adolfo (CIBERNED, San Sebastián).

Title: **Different role of microglia and astrocytes in the onset and progression of nigrostriatal degeneration.**

Authors: Quiroga-Varela A, Rodríguez-Chinchilla T, Molinet-Dronda F, Blesa J, Trigo-Damas I, Pineda J, Belloso-Iguerategui A, López-González N, Gago B, Obeso JA, Rodríguez-Oroz MC.

Abstract: Neuroinflammation has been implicated in the physiopathology of Parkinson's disease (PD). Microglial and astrocyte activation is observed in the substantia nigra pars compacta (SNc) and striatum of PD patients and animal models of parkinsonsim but their role in the beginning and progression of PD is unknown. Our aim was to study the relationship between neuroinflammation and nigrostriatal degeneration in two models of progressive parkinsonism: one induced by overexpression of mutated human ?-synuclein (?-syn) in the SNc in rat and other by progressive intoxicated MPTP primate. In these models, in vivo microglia activation studied by [18F]-DPA714 positron emission tomography (PET) and post mortem molecular markers of neuroinflammation and dopaminergic lesion have been assessed at different time points of disease progression (24h, 1, 2, 3 and 16 weeks in rats and 2, 6 and 14 weeks in primates). PET results showed in rodent model an increase of microglial activity in the SNc compared to striatum in the lesion group in all time points (24h and 3 weeks p <0.001). Accordingly, PET results showed in all MPTP monkeys an increase of microglial activity in the midbrain and in the striatum at 2 weeks after MPTP injections. This increase was reduced to normal levels at 6 weeks and 14 weeks in both presymptomatic and symptomatic monkeys. Comparison between 3 weeks and 24 h in the rodent lesioned animals showed that at 3 weeks, a slight non-significant dopaminergic depletion in SNc (30%) and a reduction in striatal TH+ immunoreactivity (p <0.001) along with a higher expression of human ?-sin (p <0.001). The Iba-1 microglial marker was increased in the SNc (p <0.001) and decreased in the striatum (p <0.001) whereas, a decrease in GFAP + astrocytes was observed in both the SNc and striatum (p <0.001). Topic: Neuronal excitability, synapses and glia: cellular mechanisms; disorders and nervous system repair.

POSTER 47

Principal Investigator: López de Munain Arregui, Adolfo (CIBERNED, San Sebastián).

Title: **Hypometabolism in orbitofrontal and perirhinal cortex is associated with impulse control disorder in Parkinson's disease.**

Authors: Navalpotro-Gómez I, Molinet-Dronda F, Botas-Peñín A, Gasca-Salas C, Carmona M, Jiménez-Urbieta H, Delgado-Alvarado M, Quiroga-Varela A, Rodriguez-Oroz MC.

Abstract: A substantial subset of Parkinson's disease (PD) patients suffers from impulse control disorders (ICD) associated with chronic dopaminergic replacement therapy1. Previous metabolic/ functional neuroimaging studies exhibit a differential activation of reward-related cerebral areas but their methodological heterogeneity makes it difficult to provide firm conclusions2,3. Objectives. Our aims are (I) to elucidate the cerebral metabolic correlates sub-serving the clinical expression of ICD in PD and (II) to evaluate the association between the cerebral metabolism, the ICD severity and the striatal dopaminergic denervation. Methods. 22 PD-ICD patients according to the QUIP-RS scale, and 19 healthy controls (HC) underwent [18F]-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) in 'on drug' state in resting condition. 16 PD-ICD patients also underwent [123I]-FP-CIT SPECT (DaTscan) to measure presynaptic striatal dopamine transporter (DaT) availability in predefined regions of interest (ROIs): ventral striatum, putamen and caudate. Correlations between regional FDG uptake and QUIP-RS as well as DaT Binding Ratios (BR) and FDG uptake have been corrected for age, gender and UPDRS-III and have been assessed using SPM8. Results. As PD-ICD patients [19 male, 3 female; 59.9±8.7 years old; 8.5±4.2 years of disease evolution; UPDRS ON motor score 19.2±10.8; Daily LEDTOTAL 1449.1mg±599.3] differed from healthy controls (HC) [10 male, 9 female; 68. ±3.2 years old] in age and gender, we included them as covariates for comparisons. PD-ICD patients exhibited lower FDG uptake bilaterally in the orbitofrontal cortex (OFC), and to a lesser extent in the left perirhinal cortex than HC without any region of hypermetabolism. Indeed, we found a negative correlation between FDG uptake and the QUIP-RS score in the left medial orbitofrontal/ prefrontal cortex. On the other hand, DaT BR in ventral striatum positively correlated with FDG uptake in bilateral SMA, the frontal eye field, the anterior prefrontal cortex, the orbitofrontal cortex, bilateral entorhinal cortex and the anterior cingulate cortex. Conclusions. This study shows that there is a bilateral hypometabolism in stimulus-reward association areas in PD-ICD patients compared to HC in resting condition, which is negatively correlated with the severity of this behaviour disorder. Higher dopaminergic denervation in ventral striatum is associated with lower cerebral metabolism in several areas of the limbic circuit This finding indicates that higher denervation in ventral striatum can predispose to functional abnormalities in the reward limbic circuit in PD patients chronically treated with dopaminergic drugs. References. 1. Weintraub D, Koester J, Potenza MN, et al. Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. Arch Neurol 2010; 67:589-95 2. Van Eimeren T, Pellecchia G, Cilia R, et al. Drug-induced deactivation of inhibitory networks predicts pathological gambling in PD. Neurology. 2010;75:1711-6 3. Voon V, Pessiglione M, Brezing C, et al. Mechanisms underlying dopamine-mediated reward bias in compulsive behaviors. Neuron. 2010 14;65:135-42.

POSTER 49

Principal Investigator: Rábano Gutiérrez, Alberto. (CIEN Foundation, Madrid).

Title: **Is it possible to distinguish primary age-related tauopathy from Alzheimer's pathology as a combined entity?**

Authors: González-Álvarez V, López-Motos D, Rábano-Gutiérrez A.

Abstract: Introduction: PART (Primary age-related tauopathy) as a distinct entity is under discussion due to its resemblance to Alzheimer's pathology. In this study we have applied the classification criteria for PART to a series of brain bank cases with predominant neurodegenerative and vascular pathologies. Our aim was to determine whether PART can be identified as a distinct combined pathology. Materials and Methods: A total 130 brains with Braak stage I ≤ IV were classified either as PART(+) (Thal Aβ phase ≤ 2 ; n = 53) or as PART(-) (Thal Aβ phase > 2 ; n= 77). Demographic, macroscopic, histopathological and diagnostic features were compared between groups. The neuropathological profile for phospho-tau inclusions (AT100 antibody) was additionally assessed in a subgroup of cases. Genetic analysis for APOE and MAPT H1/H2 was performed in a limited number of cases. Results: Age at death is lower for PART(+) cases (71, 31 ± 9,8 yrs vs. 80,30 ± 8,5 yrs; p< 0,05). APOE ε4 and MAPT H1 status did not differ among groups. An association was observed between PART(-), Alzheimer's, Lewy and vascular pathology while PART(+) features were predominant in cases with Huntington and TDP pathology, though no significant association was found. PART(+) and PART(-) brains displayed a common pattern of regional distribution and intensity of neurofibrillary pathology, and both groups form a continuum of tau (+) pathology. After stratification for Braak stage, no difference was observed between groups for regional intensity of pathology. No neuritic plaques were observed in definite PART (+) cases. Conclusion: In a brain bank series of cases with high age at death and predominant neurodegenerative and vascular pathologies, cases fulfilling current criteria for PART did not show a distinct demographic, pathological or genetic profile. Accordingly, in our series PART seems to form a continuum with conventional Alzheimer's tau pathology.

POSTER 50

Principal Investigator: Rábano Gutiérrez, Alberto. (CIEN Foundation, Madrid).

Title: **Factors associated to disease duration in Alzheimer's patients: a clinicopathological study.**

Authors: Buendía García I, del Ser-Quijano T, Rábano-Gutiérrez A.

Abstract: Introduction: Length of survival and progression rate is poorly understood in Alzheimer's disease (AD). Diverse progression rates have been related to different phenotypes of cortical atrophy, and to the type of combined pathology (e.g. Lewy bodies or vascular lesions). The aim of this study is to analyze this variability and associated factors in a cohort of demented patients from the brain donation program at Alzheimer's Center Queen Sofía Foundation. Materials and Methods: Ninety two patients with detailed postmortem neuropathological study and relevant Alzheimer's type pathology were included. Three groups of disease duration (DD) were defined: short, ≤7 yrs. (sD, n=20); intermediate, 7-11 yrs. (iD, n=29); and long, ≥12 yrs. (lD, n= 43). Sociodemographic, clinical, cognitive, functional, neuropathological and genetic data were compared among groups through multivariant statistics. Results: Although mean age at death was not significantly different between groups, a younger age of onset was associated to longer DD. Shorter times to reach GDS=7 or Semantic Fluency Test=0 were associated to shorter survivals. No difference was observed between groups in vascular risk factors, cause of death or number of medical comorbidities. Groups did not differ either in macroscopic findings. Among variables defining Alzheimer's and common combined pathologies, only the intensity of amyloid angiopathy, the presence of hippocampal sclerosis and the stage of TDP-43(+) pathology were associated to longer DD. Conclusion: In our cohort of pathologically diagnosed AD patients the DD is determined neither by core features of Alzheimer's pathology nor by the main clinical, cognitive or functional traits. The main determinant of DD seems to be age at onset of clinical disease. Alzheimer's disease shows a homogeneous pattern of evolution over a time period whose duration is age dependent.

POSTER 52

Principal Investigator: Bullido Gómez-Heras, Mª Jesús. (CIBERNED, Madrid).

Title: **Involvement of the Lysosomal pathway in neurodegeneration induced by oxidative stress and HSV-1 infection.**

Authors: Kristen H, Llorente P, Sastre I, Recuero M, Aldudo J, Bullido MJ.

Abstract: Sporadic Alzheimer's disease (AD) is a highly complex disorder for which neither the causal agent(s) nor the molecular mechanisms behind are well known. Among the environmental risk factors, persistent brain infections, particularly those induced by Herpes simplex virus type 1 (HSV-1), seem to play a key role in the pathogenesis. Another factor is oxidative stress (OS), intimately linked to aging and, therefore, thought to be crucial to the development of the disease. Our group works with both factors to simulate sporadic AD in vitro, with the aim of identifying pathways associated with AD pathogenesis. Gene expression and functional studies of the human neuroblastoma cell line SK-N-MC showed that the interaction of OS with HSV-1 affects lysosomal function, in line with previous reports on the role of lysosomal function in early stages of AD neurodegeneration. We thus focused on the lysosomal pathway and found that HSV-1 infection and OS led to an increase of lysosomal content, decreased activity of several lysosomal hydrolases, accumulation of intracellular cholesterol, confirming that the lysosomal pathway is severely impaired. The lysosomal-associated membrane protein 2 (LAMP2) gene, claimed to be involved in the export of cholesterol out of lysosomes and in the final stages of autophagy, was one of the most strongly modulated ones, so it a strong candidate to mediate the lysosomal alterations observed in our models. Indeed, the case/control studies revealed LAMP2 genetic variants to be associated with AD risk suggesting that LAMP2 is involved in the disease. To study the role of this candidate in the neurodegenerative events induced by HSV-1, human neuroblastoma cell lines with a stable knockdown for LAMP2 were generated in-house and two additional murine LAMP2-deficient cell lines—MEFs and N2a—were used. LAMP2 deficiency induced a less effective HSV-1 infection in both cell lines, suggesting a functional role of LAMP2 in viral cycle and, consequently, in the neurodegeneration events induced by the virus.

POSTER 53

Principal Investigator: Sáez Valero, Javier. (CIBERNED, Elche, Alicante).

Title: **Acetylcholinesterase expression levels in Alzheimer's. Role of the Processing of the proline-rich membrane anchor by gamma secretase.**

Authors: García-Ayllón MS, Campanari ML, Navarrete F, Ginsberg SD, Manzanares J, Alom J, Tsim K, Sáez-Valero J.

Abstract: Alzheimer's disease (AD) is characterized by a decrease in the enzymatic activity of the enzyme acetylcholinesterase (AChE). AChE exists as different splicing variants with particular regional, cellular, and subcellular locations that may reflect differential physiological roles. We have recently demonstrated that a prominent pool of enzymatically inactive AChE protein exists in the AD brain, so we aimed to study the expression of AChE variants in Alzheimer's disease brain. Protein and transcripts levels of AChE variants were analyzed in postmortem cerebral cortex from AD patients by Western blot using specific anti-AChE antibodies and by quantitative real-time PCR (qRT-PCR). We also investigated expression levels of the anchoring AChE subunit proline-rich membrane anchor (PRiMA-1), limiting factor for correct localization of cholinergic AChE at plasma membrane. We found similar protein and mRNA levels of the major cholinergic "tailed"-variant (AChE-T) and its anchorage subunit PRiMA-1 in cortex from AD patients and non-demented controls. Interestingly, we observed an increment in protein and transcript levels of the non-cholinergic "readthrought" AChE (AChE-R) subunits in samples of AD patients. Further, we have analysed whether the PRiMA 1 intracytoplasmic domain (PRiMA-ICD) resulting of the processing by γ-secretase promotes alterations in AChE expression. We found that overexpression of PRiMA 1 leads to a decrease in the expression of AChE-T variants, with no changes in AChE-R. This reduction was reverted by the treatment with the γ-secretase inhibitor DAPT that prevents the presence of PRiMA-ICD in the nucleus, indicating a regulatory mechanism of the cholinergic variant by its anchor subunit. In conclusion our findings reveal previously unknown expression patterns of AChE variants in AD cortex likely reflecting specific roles and/or differential regulation for each variant in AD, which may have strong implications for the re-evaluation of AChE inhibitors as therapeutic agents in dementia

POSTER 54

Principal Investigator: Sáez Valero, Javier. (CIBERNED, Elche, Alicante).

Title: **Potential of the amyloid-β precursor protein as a cerebrospinal fluid biomarker of Alzheimer's disease patients**

Authors: López-Font I, Boix CP, Zetterberg H, Blennow K, Sáez-Valero J.

Abstract: Soluble fragments of the amyloid precursor protein (APP) generated by α- and β-secretases, sAPPα and sAPPβ, have been postulated as promising new cerebrospinal fluid (CSF) biomarkers for the clinical diagnosis of Alzheimer's disease (AD). However, we recently demonstrated the presence of soluble full-length APP (sAPPf) in CSF and that all the CSF sAPP species assemble into multimeric complexes, which contribute to the underestimation of specific sAβPP species when assessed by ELISA. To circumvent this issue, we analyzed by SDS-PAGE large fragments of sAPP and their variants in the CSF from AD and control subjects, probing with specific antibodies against particular domains. Similar levels of sAPPα and sAPPβ protein were found in CSF samples from AD and controls, yet there appeared to be a shift in the balance of the sAPPf species in AD samples, with a decrease in the proportion of the lower (~100 kDa) band relative to the upper (~120 kDa) band. Similar differences were observed in the contribution of the major KPI-immunoreactive APP species. The differences reveal alterations that probably reflect pathophysiological changes in the brain.

POSTER 55

Principal Investigator: Sáez Valero, Javier. (CIBERNED, Elche, Alicante).

Title: **The alteration of ApoER2 processing by Aβ could modulate Reelin expression**

Authors: Cuchillo-Ibáñez I, Mata-Balaguer T, Ferrer I, Sáez-Valero J.

Abstract: Background: Reelin signaling contributes to synaptic transmission after binding to its receptor, ApoER2. Interestingly, in the frontal cortex of Alzheimer's disease (AD) subjects there is more Reelin protein and transcripts, however, the β-amyloid peptide (Aβ) hinders Reelin's biological activity. How Aβ interferes with Reelin expression is not yet understood and it is the aim of this work. Methods: Reelin and ApoER2 transcripts were analyzed by qRT-PCR in brain extracts of frontal cortex of AD (n= 8; Braak I-II; n= 10; Braak III-IV; n= 10; Braak V-VI) and control (n= 8) brain extracts, and in 5 µM Aβ42-treated SH-SY5Y cells, a cell model that constitutively expresses all the components of Reelin signaling. The DNA methylation at cytosines in the Reelin promotor after bisulfite-modification was assayed by methylation-specific PCR and sequencing of the Reelin promoter region (−687 to −202 bp). The nuclear level of DNA-methyltransferase1 (DNMT1), an enzyme that methylates Reelin promotor therefore influencing its expression, was also estimated. Finally, the C-terminal fragments of ApoER2 (ApoER2-CTF) were assayed in Western blots. Results: We found a significant increase in Reelin mRNA in AD subjects with late Braak stages and after Aβ42 treatment in cells, while ApoER2 mRNA expression did not change. Aβ42 decreased the nuclear levels of DNMT1, however the methylation of the Reelin promoter in cells treated with Aβ42, and also in AD subjects, was not different from that of controls. By contrast, the ApoER2-CTF fragments generated after Reelin binding were found significantly diminished after Aβ42 treatment as well as in AD subjects. Conclusions: The increased levels of Reelin protein in Aβ42-treated cells and in frontal cortex of AD subjects could be explained by the decrease of ApoER2-CTF levels, and likely of ApoER2-ICD, since it is described that ApoER2-ICD down-regulates Reelin mRNA expression. Mechanisms that affect Reelin promoter methylation could not be involved.

POSTER 56

Principal Investigator: Sáez Valero, Javier. (CIBERNED, Elche, Alicante).

Title: **Rebound increase in presenilin-1 protein levels in response to γ-secretase inhibition**

Authors: Sogorb-Esteve A, García-Ayllón MS, Llansola M, Felipo V, Blennow K, Sáez-Valero J.

Abstract: Drug candidates targeting β-amyloid (Aβ) peptides generation have dominated Alzheimer's disease (AD) drug development programs for the past decades. Accordingly, targets for each individual step in this cascade have been developed, with β-secretase and γ-secretase inhibitors representing one particular opportunity for front-line therapy. Thus, γ-secretase inhibitors (GSIs) are potential therapeutic agents for AD; however, trials have proven disappointing. We addressed the possibility that γ-secretase inhibition can provoke a rebound effect, elevating the levels of the catalytic γ-secretase subunit, presenilin-1 (PS1). Acute treatment of SH-SY5Y cells with the GSI LY-374973 (DAPT), a well-known GSI that targets PS1 and reduces Aβ in vitro and in vivo, increased PS1 protein levels; yet with no increase in mRNA expression. Similar increases in PS1 were evident in primary neurons treated repeatedly (4 days) with DAPT or with the GSI BMS-708163 (avagacestat), one of the first GSI that undergone clinical trials. Likewise, rats sub-chronically administered with avagacestat (40 mg/Kg, 21 days) had more brain PS1. Sustained γ-secretase inhibition did not exert a long-term effect on PS1 activity, evident through the decrease in CTFs of APP and ApoER2. The rebound increase in PS1 in response to GSIs must be taken into consideration for future drug development.

POSTER 57

Principal Investigator: Strange, Bryan (CIEN Foundation, Madrid).

Title: **White Matter Loss in the Healthy Elderly Brain Indicative of Impending Cognitive Decline**

Authors: Zhang L, Long C, Medina M, Strange B.

Abstract: Background: Focus on early intervention in Alzheimer's disease (AD) has increased the importance of characterising its preclinical stages and identifying healthy individuals at risk of developing cognitive impairment. Whilst grey matter (GM) atrophy is a well-known biomarker in AD, evidence suggests that white matter (WM) deterioration can also predict conversion to AD from mild cognitive impairment (MCI), possibly even precede GM atrophy in AD pathophysiology. Using magnetic resonance imaging (MRI) data from the Vallecas project (a 5-year longitudinal study of healthy elderly individuals), we investigate if differences in WM density (WMD) are present in cognitively normal individuals before conversion to MCI. Methods: From a baseline cohort of 813 cognitively normal 70-85 year olds, 23 had converted to MCI by one year later. The MCI converters were matched with 23 non-converters using a novel statistical matching algorithm: each converter was matched exactly for gender and APOE genotype, then the closest match selected based on a composite score of age, years of education and MMSE at baseline. Baseline T1-weighted MRI scans of both groups were analysed using voxel-based morphometry (VBM) with SPM12 software. Segmented WM images were normalised, smoothed and entered into a two-sample t-test comparing converters and non-converters. A second analysis was conducted using the same methodology, but comparing the converters with all 790 non-converters, with matching criteria as covariates. Results: In both comparisons, converters had lower WMD than non-converters in the body of the fornix ($p<0.01$, uncorrected). The effect was stronger in the matched group comparison ($p<0.001$, uncorrected) than the unmatched, however, neither result was robust enough to survive multiple comparisons. Conclusions: Our preliminary findings suggest that WMD in the fornix may be lower in elderly individuals destined to develop MCI, prior to symptom onset. These results are consistent with other studies where WM deterioration in the fornix predicts conversion from MCI to AD. Although careful sample matching improved the result, the effects observed did not survive statistical correction, possibly as a result of early disease state or sample size. Further investigation with diffusion imaging and larger samples from the ongoing Vallecas project may provide more robust results.

POSTER 58

Principal Investigator: Fernández-Blázquez, Miguel Ángel. (CIEN Foundation, Madrid).

Title: **Neuropsychological markers are sensitive for early detection of mild cognitive impairment: results from the Vallecas project**

Authors: Fernández-Blázquez MA; Ávila-Villanueva M; del Ser T; Medina M.

Abstract: Objectives: Early detection of cognitive impairment has a paramount importance to improve health care quality of patients. The present work aims at studying the capability of a set of neuropsychological parameters in order to predict the conversion to Mild Cognitive Impairment (MCI) in a sample of older adults. Materials and methods: The participants of this study comprised 920 community-dwelling individuals aged 70 years and over (age=75.0±3.9 years; 63.4% women; education=10.5±6.5 years). All of them were part of the Vallecas Project cohort, a community-based prospective research for early detection of Alzheimer´s Disease. To be considered eligible for participating in this study, subjects had to have been diagnosed as cognitively healthy at baseline. Participants underwent annually a complete evaluation consisting of blood extraction, neurological exam, neuropsychological assessment and neuroimaging study. After each visit every participant was independently diagnosed at consensus meetings according to clinical criteria. Results: Participants were followed up for a median of 38 months (range 11-61). During this time 77 individuals (8.4% of the sample) were identified as converters to MCI. Adjusted Cox proportional hazard regression models were conducted to control for demographic, clinical and genetic variables. After adjustment, the majority of neuropsychological parameters at baseline showed significant association with conversion to MCI. Specifically, free recall of verbal information, both immediate (HR=0.90; 95%CI=0.85-0.96; p=0.001) and delayed (HR=0.78; 95%CI=0.69-0.89; p<0.001), showed the greatest sensitivity to detect MCI converters, even above clinical and genetic variables. Individuals who score below 19 in immediate or 7 in delayed free recall should be considered as high-risk and then might need special attention in terms of early interventions. Discussion: Neuropsychological parameters are sensitive enough to detect future cognitive decline in older adults. Ultimately the combination of neuropsychological data along with other clinical information increases the predictive value.

POSTER 59

Principal Investigator: Gomes, Claudio M. (Biosystems and Integrative Sciences Institute – University of Lisbon)

Title: **A new role for S100B protein in amyloid-βeta aggregation in Alzheimer's disease.**

Authors: Gomes CM, Cristóvão JS, Morris V, Fernández J, Pardon E, Steyaert J, Reif B.

Abstract: Insoluble β-amyloid peptide (Aβ) deposits formed in the synaptic milieu, chronic activation of glial cells and inflammation are consistent features in Alzheimer's disease (AD) and strong candidates for the initiation of this process. S100B is one of the numerous pro-inflammatory molecules produced by astrocytes, which is up regulated in AD and is found associated with plaques. S100B is a small dimeric protein whose structure and functional regulatory interactions with other proteins are modulated by calcium-binding through EF-hand motifs and by zinc- and copper-binding to dimer interface. These facts have prompted us to investigate co-aggregation phenomena involving S100B and Aβ.

Here we combine biochemical, biophysical and cellular approaches to show that Aβ42 undergoes a dynamic interaction with S100B within the interfacial cleft, which is favoured by calcium binding. Kinetic analysis of the microscopic mechanism revealed that S100B delays Aβ aggregation by decreasing mostly secondary nucleation due to complexation with Aβ42 monomers and oligomers. Moreover, Ca2+-S100B provokes an alteration of the Aβ42 aggregation mechanism that almost completely supresses Aβ42 fibril formation, rescuing cell viability and decreasing apoptosis induced by Aβ42 in neuronal cell cultures. These findings provide a mechanism for a novel role of S100B as novel extracellular chaperone supressing proteotoxicity in AD.

We will also report the characterization of a library of 20 nanobodies targeting S100B developed with the aim to generate biological tools to regulate the interaction between S100B and Aβ42. With this approach, we expect to generate knowledge that will translate into the potential use of S100B as a new druggable target to prevent or ameliorate protein aggreegation and inflammation across neurodegenerative diseases.

POSTER 60

Principal Investigator: Martins, Sandra (IPATIMUP/i3S, Porto)

Title: **Analysis and correlation between the genome and cerebral activity of late-onset Alzheimer's disease patients**

Authors: Martins S, Fernandeza L, Lopesa A, Taborda A, Oliveira V, Martínez M, Poyo M, Hornero R, García M, Poza J, Arenasa M, Gómez C, Pintoa N.

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to dementia in the elderly population. Differential diagnosis of dementia due to AD is, however, difficult to establish mainly in early stages of the disease, which is considered to progress in four general stages: mild cognitive decline; initial stage; moderate decline; and severe decline. At the genetic level, AD seems to be highly complex, with several studies attempting to characterize the mosaic of genetic contributors. The apolipoprotein E (APOE) gene has been the first susceptibility locus for late-onset AD (LOAD, observed in the vast majority of patients). Currently, APOE ε4 allele is still the most important genetic risk factor associated with LOAD, while recent studies point to multiple low penetrance genetic variants. In this project, our aims are (1) to identify novel LOAD candidate genes; (2) to characterize the population regarding coding and regulatory variants within genes at the previously identified LOAD loci; (3) to associate different cerebral activities to each of the four disease stages; and (4) to correlate variants in candidate genes and disease progression (assessed by neuroimaging). We will analyse a total of 200 LOAD patients from North Portugal (n=100) and from the Spanish community of Castile and León (n=100), previously selected according to their stage of the disease (25 patients from each group). In addition, 50 controls from both regions will also be analysed. DNA will be extracted from saliva and buccal swab samples. Cerebral activity will be assessed by electroencephalography (EEG). By correlating EEG patterns and the four stages of the disease, we expect to find a noninvasive approach to assess early stages of cognitive decline, and improving the accuracy of diagnosis. This project will be carried out by a multidisciplinary team of biologists, physicists, mathematicians, nurses and psychologists, aiming at a broader study of the disease.

POSTER 61

Principal Investigator: Oliveros Cid, Antonio (Neuropolis Foundation, Zaragoza).

Title: **Apathy effect on diagnosis in Alzheimer's disease patients.**

Authors: Oliveros-Cid A, Oliveros-Juste A, Cid-López MA, Sierra-Martinez E, Jaime-Fregenal P, García-Hojas S, Fayed N, Barrena R, Delgado-Cimorra B, Bernad-López J.

Abstract: Background: Apathy and agitation influence daily living and care in dementia. We think it can also influence diagnosis, particularly in early stages. Purpose: to examine apathy among symptoms, and relationship with referral schedule and early diagnosis. Methods: Outpatients referred for possible cognitive impairment included in a database of "apathy and cognitive impairment". Clinical history, complete neuropsychological battery, MRI, MRS, and lab test were also performed. Besides, some patients undergo also EEG exams, CSF profile and/or Amyloid-PET.

76 were finally diagnosed as Alzheimer Disease: 27 with CDR-0,5, 30 with CDR-1, and 19 CDR-2 included. Besides other cognitive batteries, apathy and behavioral symptoms were widely assessed, including Structured Clinical Interview for apathy (SCIA), Skarkstein apathy scale, Marin apathy scale, Dementia Apathy Interview and Rating (DAIR), Neuro-Psychiatric Inventory (NPI), Hamilton-D scale and Cornell Scale for Depression in Dementia (CSDD). Pharmacological treatment was also recorded. We also performed a semistructured qualification of apathy severity when patients were first referred to general practitioners (GPs). Correlations and group comparisons conducted. Results: Group differences found according to the frequency and severity of apathy in patients. Those who showed apathy or depression (Independently) as a predominant symptom when referred to GPs were more frequently either treated with psychopharmacological therapy by GPs or sent to Psychiatry, where usually AD was less taken into consideratio. Apathy level showed negative correlations with MMSE, for the same group (CDR) of patients. Significantly higher apathy levels associated with intake of antidepressants and cognitive enhancers. Conclusions: Different patterns of apathy can be observed in outpatient MCI and dementia patients; this apathy has a significant negative effect on the early neurological evaluation of those patients, thus having a deleterious effect on their opportunities for a correct approach in early stages of disease. Further analyses are needed in bigger groups of population.

POSTER 63

Principal Investigator: Oliveros Cid, Antonio (Hospital Reina Sofia. Tudela).

Title: **CSF and neuropsychological profile differences between normal pressure hydrocephalus and Alzheimer's disease patients.**

Authors: Oliveros-Cid A, Oliveros-Juste A, Cid-López MA, García-Hojas S, Sierra-Martínez E, Jaime-Fregenal P, Antón-Aguirre S, Jarauta-Salvador F, Barrena R, Chueca-Rodríguez P.

Abstract: Background: Idiopathic normal pressure hydrocephalus (iNPH) is a complex and still underestimated pathology. In the early stages, the cognitive profile is characterized mainly by impairments of attention, psychomotor speed and memory, suggesting frontal involvement; patients with more advanced iNPH show overall cognitive deterioration. The memory impairment, however, seems to be milder than that seen in Alzheimer's disease (AD). Clinical and neuroimaging data are crucial for the diagnosis of iNPH, but the presence of different variables, such as comorbidities, and the possible overlapping with other neurodegenerative diseases, AD in particular, make the differential diagnosis difficult.

Material and Methods: The purpose of the present study is to identify an iNPH-specific cerebrospinal fluid (CSF) biomarker profile and to assess its ability to help neuropsychological profile to differentiate iNPH from AD. Total tau (t-tau), tau phosphorylated (p-tau), amyloid-β (Aβ) 42 and Neuronal Specific Enolase (NSE) were measured in 33 consecutive CSF samples consisting of 13 iNPH (8 tap test responders) and 20 AD patients. Levels of t-tau and p-tau were significantly decreased in iNPH patients especially in tap test responders compared to AD. More correlation was observed between Mini-Mental State Examination scores and Aβ42 in AD than in iNPH. NSE levels did not differ between the two. Conclusion: besides Neuropsychological profile and tap test respond, CSF t-tau and p-tau can be useful for differentiation of iNPH and AD.

POSTER 64

Principal Investigator: Oliveros Cid, Antonio (Clínica Sagasta - Creu Blanca. Zaragoza).

Title: **Effect of cholinesterase inhibitors therapy in patients with depression and cognitive impairment.**

Authors: Oliveros-Cid A, Cid-López MA, Oliveros-Juste A, Jaime-Fregenal P, García-Hojas S, Sierra-Martinez E, Pagola-Lorz I, Fayed N.

Abstract: Background: In patients with comorbid depression and cognitive impairment, antidepressants are commonly used but the utility of acetyl-cholinesterase inhibitors (AChel) as cognitive enhancers is not established. Besides, some of those patients receive AChel because of "cognitive impairment", without a clear diagnosis of dementia. Methods: 25 patients with depression (55-87 years old) referred as "cognitively impaired". They had never started therapy with AChel (donepezil, galantamine or rivastigmine) or memantine. We analyzed for cognitive impairment (complete neuropsychological and neurobehavioral battery, MRI, MRS, lab test, ApoE), and 14 of 25 patients met criteria for amnestic mild cognitive impairment (MCI). After 3 month stable AChel therapy, all patients underwent again a complete neuropsychological battery. Results: there were a slight increase in neurocognitive performance in those with amnestic mild cognitive impairment compared with those with those with non-amnestic cognitive impairment. Both groups improved similarly in depressive symptoms, as measured by neuropsychological tests and caregiver oriented tests Conclusions: In patients with comorbid depression and cognitive impairment, the addition of AChei following antidepressant medication treatment does not improve cognition compared to placebo in non-amnestic cognitive impairment type or those with cognitive impairment due to depression. Although the study was performed in a small but very homogeneous group of patients, we can conclude that presence of depression and response to antidepressant medication does not have to delay AChel therapy in AD suspected patients.

POSTER 65

Principal Investigator: Waites, Clarissa (Columbia University, New York City, NY)

Title: **Implication of RAB35/ESCRT pathway in tau proteostasis and its impact on the stressed brain.**

Authors: Waites C, Sotiropoulos I.

Abstract: Chronic stress and excessive glucocorticoid (GC) exposure are suggested to increase susceptibility to brain pathology as they are associated with neuroplastic deficits and impaired cognition. In line with clinical evidence suggesting chronic stress as risk factor of Alzheimer's disease (AD), experimental studies demonstrated that stress and GC trigger AD-related pathomechanisms, including the intracellular accumulation of hyperphosphorylated Tau protein, suggesting that dysregulation of Tau proteostasis is a major causative factor for stress-induced brain pathology. However, the mechanisms underlying Tau turnover and degradation are still poorly understood. Here, we have identified a novel molecular pathway that mediates the degradation of Tau in hippocampal neurons. This pathway comprises the small GTPase Rab35 and the endosomal sorting complex required for transport (ESCRT) machinery, which catalyzes the biogenesis of multivesivular bodies (MVBs) for delivery of cargo to lysosomes. Interestingly, we find that the Rab35/ESCRT pathway is negatively regulated by GC, which are known to impair Tau degradation and lead to Tau accumulation and synaptic missorting. Furthermore, we show that stimulating this pathway via Rab35 overexpression can rescue GC-induced Tau accumulation, thus supporting the relevance of the Rab35/ESCRT pathway for Tau pathomechanisms, and identifying a promising therapeutic target for the treatment of stress-related neurological pathologies, including AD.

POSTER 66

Principal Investigator: Guimas Almeida, Cláudia (CEDOC - NOVA Medical School, Lisboa).

Title: **Mechanisms of beta-amyloid endocytic production in late-onset Alzheimer's disease.**

Authors: ND.

Abstract: Late-onset AD (LOAD) affects 1 in 10 elderlies over 65 years old. AD is a progressive neurodegenerative disease that impairs memory, behavior, and ability to be independent.

Early-onset familial AD (eFAD) is rare and caused by mutations in amyloid precursor protein (APP) or presenilins (γ-cleavage of APP) that lead to excessive neuronal production of the longest form of beta-amyloid (Aβ42) or increased ratio of AB42 over Aβ40. Mouse models carrying eFAD mutations recapitulate cognitive memory deficits and develop amyloid plaques and tangles. APP processing occurs in endosomes suggesting that intracellular Aβ42 generation contributes to the initial Aβ42 accumulation in eFAD. In LOAD the causal mechanisms are multifactorial and less clear. Genome wide association studies (GWAS) of thousands of LOAD patients were undertaken and among the identified genes at highest risk in AD are Sorl1, BIN1, PICALM and CD2AP, regulators of endocytic trafficking. APP temporal and subcellular localization is different from its secretases. Evidence supports that endocytic trafficking of APP determines encounter with β- and γ-secretases. We are investigating the mechanisms whereby Bin1 and CD2AP are interfering with APP and BACE1 endocytic trafficking. I will present our recent data on how their loss of function increases Aβ42 generation by surprisingly specific and polarized mechanisms (Ubelmann, et. al, 2017). I will give an update on our current research on the impact of AD variants in CD2AP and Bin1 in endosomal amyloid production. We expect to determine if individuals carrying variants of either factor would slowly accumulate Aβ42 in neurons increasing the risk for late-onset AD.

POSTER 67

Principal Investigator: Miranda, André (Columbia University Medical Center, New York City, NY).

Title: **Neuronal lysosomal dysfunction releases exosomes harboring app c-terminal fragments and unique lipid signatures.**

Authors: Miranda A, Lasiecka ZM, Xu Y, Neufeld J, Shahriar S, Simões S, Chan RB, Gil-Oliveira T, Small SA, Di Paolo G.

Abstract: Defects in endolysosomal and autophagic functions are increasingly viewed as key pathological features of neurodegenerative disorders. Indeed, Alzheimer's disease (AD) and a variety of other disorders are now seen as late onset, milder versions of lysosomal storage disorders, which are typically very aggressive monogenic diseases. A master regulator of these functions is phosphatidylinositol-3-phosphate (PI3P), a phospholipid synthesized primarily by class III PI 3-kinase Vps34. Following up our previous lipidomic study showing a selective reduction in PI3P in the brain of patients with AD as well as in mouse models thereof, our study exploits pharmacological and genetic tools to manipulate the Vps34 pathway. Here we report that disruption of neuronal Vps34 function in vitro and in vivo impairs autophagy, lysosomal degradation as well as lipid metabolism, causing endolysosomal membrane damage. PI3P deficiency also promotes secretion of unique exosomes enriched for undigested lysosomal substrates, including amyloid precursor protein C-terminal fragments (APP-CTFs), specific sphingolipids and the phospholipid bis(monoacylglycero)phosphate (BMP), which normally resides in the internal vesicles of late endosomes and lysosomes. Secretion of these exosomes requires neutral sphingomyelinase 2 and is partially mimicked by lysosomal alkalization. Our results reveal a specific homeostatic response counteracting lysosomal storage disorder via secretion of atypical exosomes eliminating lysosomal waste and define exosomal APP-CTFs and BMP as candidate biomarkers for endolysosomal dysfunction associated with neurodegenerative disorders.

POSTER 68

Principal Investigator: Teixeira, Sara (Centro Hospitalar Lisboa Ocidental).

Title: **Promotion of mental health in the elderly.**

Authors: ND.

Abstract: As a risk factor of generational progress, age accelerates the appearance of Neurodegenerative Diseases, with political-economic consequences for the Health Service and the quality of life of the population. It increases the importance of early intervention among the elderly population. Thus, neuropsychological assessment is one of the most important complementary diagnostic tests. By allowing discrimination normative declines, the identification of initial cognitive decline, dementia, emotional disorders or medical disorders. The neuropsychological evaluation is composed of a semi-structured interview, where is identificated personal and clinical history; medical history; family and social history; academic/professional history. And a battery of evaluation instruments, validated and standardized for the Portuguese population, what is constituted by the Montreal Cognitive Assessment Test (MoCA) that assesses visuospatial capacity, executive functions, language, short-term and deferred memory, temporal and spatial orientation, attention, concentration and working memory; The Clock Drawing Test (TDR) that evaluates comprehension, memory, visuopercetive and visuomotor processes, executive functions, attention and concentration, abstract thinking, symbolic representation, graphic ability and frustration tolerance; The Wechsler Intelligence Scale for Adults (WAIS-II), sensitive to functions such as attention, concentration and working memory, knowledge general information, visual memory, learning ability; The Complex Geometric Figures Memory Copy and Reproduction Test (FCR) capable of evaluating visual memory, visuospatial capacity, perceptual organization, executive Functions (action planning, working memory, flexibility), comprehension, learning and symbolic representation; and The Raven Standard Progressive Matrices (MPS) which is a measure of general intelligence. According to statistical data collected by the Community team of the Service of Psychiatry and Adult Mental Health of CHLO-EPE, most of the examined have cognitive deficits, highlighting changes in Immediate and Deferred Visual Memory, Immediate and Deferred Verbal Memory, Executive Functions, Perceptual Organization and Work Memory. The commitment to these cognitive functions interferes with and impairs the regulation of behavior, decision-making processes, and the capacity for understanding and judgment.

POSTER 69

Principal Investigator: Diógenes, María José (Instituto de Medicina Molecular, University of Lisbon).

Title: **Protection of BDNF receptor cleavage as a possible therapeutic strategy for Alzheimer's disease.**

Authors: Fonseca-Gomes J, Jerónimo-Santos A, Sebastião AM, Diógenes MJ.

Abstract: Alzheimer's Disease (AD) is the most common form of dementia worldwide and the accumulation of amyloid-beta (Aβ) peptide in the brain is considered a main hallmark of this disease. In AD, Brain-derived neurotrophic factor (BDNF) signalling is seriously impaired, compromising its physiological functions: neuronal survival, differentiation and plasticity. Actually, decreased levels of BDNF and its TrkB Full-Length receptor (TrkB-FL) were described in several pathologies including AD. It is also widely known that there is an upregulation of TrkB truncated isoforms, which act as negative modulators of BDNF signaling. Recently, we described that accumulation of Aβ peptide leads to calpain overactivation, through the increase of intracellular calcium levels mediated by eNMDAr, and subsequent TrkB-FL cleavage. As consequence, TrkB-FL levels decrease and two fragments are generated: a membrane-bound truncated receptor (TrkB-T') and an intracellular fragment (TrkB-ICD). Accordingly, this work aimed to characterize the TrkB-ICD fragment and its consequences on cell signalling. Experiments, performed in human neuroglioma cell line and in primary rat cortical neurons, revealed that: i) TrkB-ICD is a stable fragment (half-life time ~8h); ii) it accumulates within the cell nucleus overtime and iii) it has tyrosine kinase activity, phosphorylating nuclear, somal and axonal proteins. Moreover, attending to the extremely relevant role of BDNF signalling for the endogenous neuroprotection, we performed structural predictions, using PEP-FOLD software, and designed a specific peptide to act as a substrate for calpains (TAT-TrkB) in order to study whether the prevention of calpains mediated cleavage of TrkB-FL could be a good strategy to prevent the loss of BDNF functions. Taken together these data suggest that TrkB-ICD could have a role on the pathophysiology of disorders where calpains are overactivated, such as in AD. Moreover, our structural prediction of TAT-TrkB will allow us to further prove the importance of TrkB-FL cleavage prevention on pathologic contexts.

POSTER 70

Principal Investigator: Israely, Inbal (Champalimaud Research, Lisbon)

Title: **Homeostatic plasticity induces structural scaling and selective threshold modulation of single dendritic spines.**

Authors: Israely I, Hobbiss A.

Abstract: Neural networks employ homeostatic synaptic plasticity (HSP) to maintain activity within an optimal range, countering tendencies of unchecked LTP or LTD to saturate the network. HSP is manifested by synaptic scaling of all the inputs on a neuron, either upwards to increase global activity or downwards to decrease it. We investigate whether this form of plasticity leads to long lasting structural changes at spines, and whether such modifications impact the ability of neurons to undergo futher plasticity, and thus encode information, at single inputs. We find that prolonged activity blockade causes structural growth of individual dendritic spines of hippocampal CA1 neurons, complementing physiological increases in synaptic strength. This volume increase occurs across the population of spines and can be reversed after the activity block is lifted. To determine how structural homeostatic plasticity affects encoding of information at single inputs, we induce glutamate uncaging mediated long term potentiation at select spines following activity blockade, and optically monitor changes. While such spines still undergo structural potentiation, the strength of plasticity is differentially modulated depending on the initial volume. Smaller spines show a more robust and long lasting growth of those inputs compared to size matched controls, whilst large spines do not undergo any significant structural synaptic plasticity. Interestingly, we find that following HSP, neighbouring spines in the vicinity of a stimulated input undergo transient growth. This contrasts with the input specificity seen under normal conditions whereby only the stimulated spine shows structural plasticity. HSP thus leads to metaplasticity by inducing a spread of structural plasticity beyond a single synapse. Overall, these findings illustrate that whilst the network retains information-coding capacity following homeostatic plasticity, the optimal strength of the network is regulated through the preferential modulation of smaller synapses; whilst larger ones, which presumably contain previously learned information, remain stable.

POSTER 71

Principal Investigator: Gama Correia Malta Vacas, Sara (Champalimaud Research, Lisbon)

Title: **Gertmann syndrome as a inicial presentation of Alzheimer's disease.**

Authors: Gama-Correia S, Malta-Vacas S, Oliveira-Maia A.

Abstract: Gerstmann Syndrome (GS) is a rare neurological condition described as a group of cognitive changes corresponding to a tetrad of symptoms comprising agraphia, acalculia, right-left disorientation and finger agnosia. It is known that some specific brain lesions may lead to such findings, particularly when there is impairment of the angular gyrus and adjacent structures.

We aim to report a case of a young, psychiatric patient who presented with cognitive complains and clinical symptoms compatible with the tetrad of GS.

A 49 years old right-handed female with university school education with no medical history was initially brought to the emergency room department by his family because of worsening of recent memory, executive function, and mixed anxious-depressive mood. She had a 20 years old history of personality disorder with frequent psychiatry outpatient consultations. This patient displayed all four symptoms of GS. In addition to those symtoms, neuropsychological evaluation revealed deficits on attention, episodic memory and visuospatial abilities. The cerebrospinal fluid evaluation showed a moderate increase in CSF P-tau and a marked decrease in CSF Aβ42. Diffuse cortical atrophy, most predominant on the parietal and frontal lobes lobe, was visualized by brain MRI. She was diagnosed with probable early onset Alzheimer's Disease.

We report a case of a 49 years old woman with previous psychiatric history which presented with GS and cognitve decline and mood complains who was diagnosed with probable early onset Alzheimer's Disease.

POSTER 72

Principal Investigator: Marques dos Reis, Mariana (Champalimaud Research, Lisbon)

Title: **The role of cell competition in drosophila's model of Alzheimer's disease.**

Authors: Marques-dos Reis M, Reis M, Moreno E.

Abstract: Affecting more than 44 million of people worldwide, with a prevalence of 1 in 7 men and 1 in 5 women, Alzheimer's disease (AD) is one of the biggest social and scientific challenges nowadays. The condition is the result of the accumulation of amyloid-beta plaques extracellularly in the brain and intracellular neurofibrillary tangles which are aggregates mainly constituted by tau protein.

Several models have been constructed to mimic the disease phenotype and Drosophila melanogaster is widely used due to its simplicity and genetic similarities to the human genome. In Eduardo Moreno's group, we use models of neurodegenerative diseases to study the role of cell competition in a disease context. In homeostatic conditions, cells can compare their fitness status by the expression of the flower fingerprints. Less fit cells express Flower lose isoforms that mark them as losers and, if the surrounding cells exhibit better fitness conditions, azot – a cell fitness checkpoint - activates a cascade of events leading to their apoptosis.

The expression of flower and azot in AD flies expressing amyloid-beta was already studied by the group, showing that elimination of unfit neurons by this mechanism is beneficial -ameliorating the phenotype of the disease- in terms of locomotion, memory and brain morphology.

We are now studying cell competition in flies expressing a human form of tau in the eye, the central nervous system, and cholinergic neurons. The main objective is to have concordance with the Amyloid-beta results and, in this way, consolidate the theory that cell fitness is a neuro protective mechanism with potential therapeutical value.

POSTER 73

Principal Investigator: Costa, Rui M (Champalimaud Research, Lisbon)

Title: **Assessment of movement sequence kinematics in dopamine depleted animals.**

Authors: Costa RM, Mendonça M, Alves-da Silva J, Hernández L, Obeso JA.

Abstract: Background: Basal ganglia function models frequently focus on initiation deficits. This contrasts with what is observed in Parkinson's Disease (PD) where chronic dopamine depletion leads not only to "slowness of initiation" but also to reduction in speed and amplitude of repetitive actions (Bradykinesia). It seems reasonable to hypothesize that dopamine has a pivotal role on movement speed.

Methods: Repetitive finger tapping is clinically used to assess PD patients. Using this as an inspiration we developed a new self-paced operant task, in which mice learn to perform a sequence of actions, using only one forelimb. We collect data on spatial position, speed and acceleration of the mouse forelimb and lever. A miniature epifluorescence microscope (~1.9g) is used to image GCaMP6f fluorescence (a calcium indicator) in dopaminergic Substantia Nigra pars compacta (SNpc) cells while TH-cre mice performed the task. After animals learned the task partial dopamine depletion is induced by unilateral intrastriatal 6-Hydroxydopamine injection.

Results: With our task we were able to assess with high spatial and temporal resolution the movement kinematics of unilateral forelimb lever-press. The presses speed correlates with task-relevant features (Number of rewards obtained, number of lever presses/sequence) but not with the total number of lever presses/session.

After dopamine depletion we found an increase in slower movements, longer within-sequence inter-press intervals and shorter sequences.

Phasic activity of SNpc dopaminergic cells accompanies the start of a learned lever-press sequence both in healthy and partially dopamine depleted animals.

Conclusion: We developed a clinically-relevant task for movement sequence kinematics assessment in mice, and identified SNpc correlates of movement. Ongoing analysis using these 2 tools will allow us to clarify the role of SNpc dopaminergic neurons in different type of movements (slow vs. fast movements), in healthy and chronic dopamine depleted mice. This will increase our understanding of basal ganglia dysfunction in PD.

POSTER 74

Principal Investigator: Cruz, Ana V. (Champalimaud Research, Lisbon)

Title: **The tectonigral projection modulates initiation of locomotor activity?**

Authors: Cruz AV, Afonso AR, Costa RM.

Abstract: The dopaminergic system is thought to modulate gait initiation, but the extent and specific role of its involvement is still unclear. Our lab has shown that movement initiation is preceded by a transient increase in dopaminergic neural activity in the Substantia Nigra pars compacta (SNc) [Alves da Silva, et al., submitted]. The Superior Colliculus (SC), which has been identified as one of several brain regions with descending projections to the SNc [Comoli et al., Nat Neurosci, 2003], has been implicated in spatial orientation. This innate behavior consists of a coordinated set of movements that allow the organism to navigate or fixate an object. Considering that orientation and gait initiation/directed locomotion are intrinsically coupled, we are particularly interested in learning how the neural activity in the deeper layers of the SC, in particular from the tectonigral projection neurons, are linked to fine motor behavior and if its modulation interferes with locomotor strategies in navigation or self-paced exploration of an open field arena.

So far, we have identified substantial glutamatergic tectonigral projections. Using electrophysiology and optogenetic tools, we have photo-identified and recorded the activity of SC glutamatergic neurons projecting to the SNc while the animal is freely moving. By optically manipulating the tectonigral projection neurons via axonal targeting, we want to understand if the SC, through the tectonigral pathway, contributes to the increase in dopaminergic activity in the SNc as the animal orients itself while preparing to initiate gait.

POSTER 75

Principal Investigator: Obeso Inchausti, José Ángel (CIBERNED, Madrid).

Title: **Activation of medium spiny neurons by optogenetics induces dyskinesias in a Parkinson Disease model.**

Authors: Hernandez LF, Castela I, Ruiz-DeDiego I, Obeso JA, Moratalla R.

Abstract: Levodopa (L-DOPA) treatment is still associated with the development of L-DOPA-induced dyskinesias (LIDs) in the majority patients with Parkinson disease (PD), which often impairs quality of life and necessitate several therapeutic adjustments. Simultaneous optical activation of medium spiny neurons (MSN) of the direct and indirect striatal pathways in the 6-OHDA (6-hydroxydopamine) rat model of PD induced LID-like movements and induced FosB expression, a molecular marker of LIDs, primarily in the striato-nigral direct pathway. We show that generation of LIDs is not associated with opposite physiological activity of the direct and indirect pathways. Moreover, the optogenetic activation of the dorsolateral striatum suggests that this abnormal activation, despite being exclusive from the dopamine depletion state, is independent of dopamine receptor involvement. Altogether, these results are a potential breakthrough in the understanding of mechanisms and pathways involved in dyskinesias.

POSTER 76

Principal Investigator: Matute Almau, Carlos (CIBERNED, Bilbao).

Title: **Microglia reduces extracelullar amyloid but exacerbates synaptic dysfunction in Alzheimer's disease.**

Authors: Capetillo-Zarate E, Zuazo J, Ortiz-Sanz C, Alberdi E, Matute C.

Abstract: The progressive and anatomically selective accumulation of β-amyloid (Aβ) peptide and the synaptic dysfunction are the main hallmarks of Alzheimer disease (AD) neuropathology. Synaptic dysfunction is the best pathological correlate with cognitive decline, but the cellular mechanism by which Aβ may affect synapses remains unclear. Recent discoveries pointing to the key role of microglia on synapses opens new research routes in neurodegeration research. A recent publication also shows that microglia mediates early synapse loss in AD models. To study the role of microglia in the control of synapse number we performed immunofluorescence, western blot techniques to measure the levels of pre- and post-synaptic markers in neurons cultured alone or together with microglia in the presence or absence of Aβ oligomers. We performed inmuprecipitation for Aβ detection in the media. First, we found that Aβ reduces significantly synaptic marker labeling in single cultures of neurons as compared to controls, non-treated cells. We also found that microglia reduces extracellular amyloid in microglia neuron-co-cultured in the presence of extracellular Aβ oligomers. However, synaptic decrease was exacerbated when neurons were co-cultured with microglia. Overall, these results indicate that Aβ itself is capable of damaging synapses in neuronal primary culture, a feature that is aggravated in the presence of microglia. Surprisingly, even though microglia is capable of degrading extracellular Aβ, at the same time contributes to synapse loss. These results strongly suggest that Aβ oligomers are deleterious to synaptic function either by interfering directly with neurons or via microglia which further contribute to synapse loss.

POSTER 77

Principal Investigator: Rodríguez Álvarez, José (CIBERNED, Barcelona).

Title: **Characterization of Nrg1/ErbB signaling pathway in neurodegeneration mouse models.**

Authors: Javier-Torrent M, Herrando-Grabulosa M, Bosch A, Rodríguez J, Sáez-Valero J, Navarro X, Saura CA.

Abstract: Recent evidences suggest altered neuregulin-1 (Nrg1)/ErbB signaling in the neurodegenerative processes leading to Alzheimer´s disease (AD) and amyotrophic lateral sclerosis (ALS). Previous studies demonstrated that sequential processing of neuregulin-1 (Nrg1) and its receptor ErbB4 by a- and b-secretases followed by presenilin (PS)/g-secretase affects Nrg1/ErbB4/3 signaling and myelination (Fleck D. et al., 2013; Willem et al., 2006). To address the role of Nrg1/ErbB4 processing and signaling in neurodegeneration in vivo, we have characterized the expression levels of Nrg1, ErbB3 and/or ErbB4 in the cortex of presenilin1/2 (PS) conditional double knockout (cDKO) mice and SOD1G93A transgenic mice. Biochemical analyses of cortical lysates show a significant genotype-dependent accumulation of C-terminal-derived Nrg1 fragments and reduced ~40 kDa ErbB4 fragment, which can represent the ErbB4 intracellular domain, in PS cDKO mice at 6-12 months of age. In the SOD1G93A transgenic mice model of ALS, our results show an increase in the phosphorylation levels of ErbB4 (Tyr1284) at 8 weeks of age, while total ErbB4 levels both in homogenate and nuclear fractions in SOD G93A mice remains unchanged during aging. Together, these results indicate PS/g-secretase-dependent processing of Nrg1 and ErbB4 in the cortex of adult mice, and impaired Nrg1/ErbB4 processing/levels during neurodegeneration

POSTER 78

Principal Investigator: López Barneo, José (CIBERNED, Sevilla).

Title: **Effect of mitochondrial function on neural stem cell proliferation and differentiation and on neuronal survival.**

Authors: Cabello-Rivera D, Sarmiento-Soto H, López-Barneo J, Muñoz-Cabello AM.

Abstract: Mitochondrial metabolism is especially relevant in stem cell biology, regulating different processes such as quiescence, proliferation and differentiation. In this work we have studied the role of mitochondrial function in neural stem cells (NSCs). Specifically, we have analyzed how mitochondrial complex I (MCI) dysfunction affects NSCs proliferation and differentiation, and survival of their neuronal progeny. We have generated the conditional knockout model hGFAP-NDUFS2, in which expression of the NDUFS2 protein, essential for MCI function, is suppressed in cells expressing the CRE recombinase under the human GFAP (glial fibrillary acidic protein) promoter, active in astrocytes and in mouse radial glial cells. In this model we have observed that central NSC population, as well as astrocytes and neuroblasts derived from these NSCs during prenatal development do not appear to be functionally affected by MCI dysfunction. Nevertheless, NSC differentiation and subsequent maturation of neurons in the perinatal period seem to be highly dependent on the correct functioning of MCI. In addition, in vitro studies in subventricular zone (SVZ)-derived neurosphere cultures showed that cell proliferation of the intermediate progenitors is seriously compromised. On the other hand, peripheral nervous system does not appear to be altered.

POSTER 79

Principal Investigator: Strange, Bryan (CIEN Foundation, Madrid).

Title: **Persistent homology: a computational topology-based approach to study brain connectivity**

Authors: Gómez-Ramírez J, Strange B.

Abstract: A mechanistic understanding of brain function and malfunction will necessary require to establish a causal theory of the brain. Candidates for global brain theories are not missing e.g. Friston's Free Energy minimization (Fris- ton, 2010), von Malsburg's correlation theory (Malsburg, 1994), Abeles cortinomics (Abeles, 1991), Llinás thalamo- cortical loop (Llinás, 1993), Tononi's integrated information theory (Marshall, 2016) etc. but the jury is still out on a causal explanation of cognition. Neuroimaging reveals only correlations. Causality can only be investigated through intervention, that is, stimulation or lesion. This poses a tremendous challenge in a nonlinear highly coupled system like the brain. Intervention in one area of the brain can ripple to other parts in very complex and unpredictable ways. In this work we make the argument that network models fall short of producing mechanistic models of normal function and disease. Graphs are built by connecting one pair of elements at a time. The limitation of having exclusively dyadic (or bivariate in statistics jargon) relationships is a crucial limitation that is often overlooked (Giusti, 2016). Computational topology allows us to go beyond pairwise connections within an elegant mathematical framework. In particular, the connectomics of the mammalian brain can be studied with per- sistent homology. Persistent homology is a method of computational topology that studies the persistent structure in data sets. This framework provides a compact encoding of multi-scale relations and is agnostic to the way the coupling is computed (power-based, phase-based, Granger causality, dynamic time warping etc.) We will show using resting state fMRI connectivity data, how persistent homology provides new views to understand the interplay between strong and weak connections in the brain connectome.

POSTER 80

Principal Investigator: Fernández Blázquez, Miguel Ángel (CIEN Foundation, Madrid).

Title: **Neuropsychology as an early marker in Alzheimer's disease. A retrospective view of the Vallecas Project.**

Authors: Ávila-Villanueva M, Frades-Payo B, Fernández-Blázquez MA.

Abstract: Neuropsychological Assesment has been widely used in the last 30 years in the characterization of dementia associated with AD. Well stablished for identification, staging and tracking for the disease, nowadays the assessment is focused in prevention and early detection. In the Vallecas Project context, a longitudinal multidisciplinary study for early markers in preclinical stages of AD, the Neuropsychology department at CIEN Foundation has four research lines that may contribute to this purpose. In this communication we would like to share our findings and future objectives.

POSTER 81

Principal Investigator: López Barneo, José (CIBERNED, Sevilla)

Title: **Insights into the striatal parvalbumin neurons: towards a specific stimulation of GDNF to protect the nigrostriatal dopaminergic neurons**

Authors: Enterría-Morales D, López-López I, López-Barneo J, d´Anglemont de Tassigny X.

Abstract: The most disabling motor symptoms in Parkinson's disease (PD) stem from the progressive death of the nigrostriatal dopaminergic (DA) neurons. Since there are limited treatment options for PD, neuroprotective agents are currently being tested as a mean to slow disease progression. The use of glial cell line-derived neurotrophic factor (GDNF) held high expectation from preclinical studies, but its exogenous administration in clinical trials proved more problematic. Previous work from our laboratory suggests that stimulating endogenous GDNF, which is mostly produced by the parvalbumin (PV) GABAergic interneurons in the mouse striatum, may be a valuable strategy to protect the DA neurons. We are seeking a method to specifically increase striatal GDNF and assess its potential as a possible therapy for PD. Our approach is based on the molecular mechanisms that make the striatal (ST) PV interneurons the main GDNF providers, which differ from other PV neuronal population that does not produce GDNF, such as those located in the cortex (CTX). To this end, we used PV reporter mice to capture the tdTomato fluorescent ST or CTX PV interneurons by FACS and run transcriptome analysis. Gene ontology analysis revealed remarkable differences in the intracellular pathways in ST and CTX PV interneurons. Moreover, the comparison of gene expression showed unique receptors and transcription factors that are selectively expressed in PV interneurons of the striatum. These may possibly modulate the striatal GDNF production. The relevance of these specific target genes and pathways is currently under investigation, but these data bring us closer to unravel the cellular/molecular pathways that drive Gdnf expression, and to identify potential pharmacological targets to specifically stimulate striatal GDNF synthesis. The findings of this study could enable us, in the future, to tryout a neuroprotective approach in clinical trials to reduce the degeneration of dopaminergic neurons in PD.

POSTER 82

Principal Investigator: Montero Olimpio (

Title: **Plasma biomarkers for Parkinson's disease diagnosis**

Authors: Albillos S, Cubo E, Solano-Vila B, Casais S, Delgado S, Velasco M, Trejo JM , Montero O.

Abstract: Parkinson's disease (PD) is the second important neurodegenerative disease worldwide. Accurate methods for early diagnoses with minimal invasion are highly demanded. In this study, plasma samples from i) idiopathic PD patients diagnosed de novo with no dopaminergic treatment (NOVO), ii) idiopathic PD patients diagnosed and with dopaminergic treatment (ON), (iii) idiopathic PD patients diagnosed and with dopaminergic treatment but this halted for 24 h (OFF), and iv) healthy controls (C), were analyzed by ultrahigh performance liquid chromatography coupled to mass spectrometry (UPLC-ESI-QToF-MS) with the aim to find potential biomarkers for early and differential diagnoses of Parkinson's disease. Levels of □-Synuclein in the plasma samples were also measured using specific antibodies by ELISA. Data acquired by UPLC-MS were submitted to untargeted analysis by partial least-square discriminant analysis (PLS-DA) using MarkerLynx® and Extended Statistics (XS)® software. No clear groups could be distinguished in the Score-Plots when all the samples were compared, but NOVO, OFF and ON samples could be grouped separately from control samples (C) after paired comparison. Deregulation of □-oxidation pathway was evident in NOVO group as compared to the control group. Phenylalanine and antioxidant metabolisms seem to be also altered in patients as compared to controls. □-Synuclein exhibited a significantly lower content in NOVO and ON groups as compared to subjects from the control group. Correlations between □-Synuclein and the clinical parameters cognitive and motor symptoms severity were shown for the ON group. Other metabolites did also correlate with different clinical parameters like non-motor and motor symptoms severity.

POSTER 83

Principal Investigator: De Felipe Oroquieta Javier (CIBERNED, Madrid)

Title: S**ynaptology of layer II of the transentorhinal cortex in Alzheimer's Disease**

Authors: Domínguez-Álvaro M, Montero-Crespo M, De Felipe J, Blázquez- Llorca L, Alonso-Nanclares L.

Abstract: Alzheimer's disease (AD) is the main cause of dementia, accounting for 60–80% of cases. It is characterized by a progressive and persistent decline of superior cerebral functions, such as memory. During the course of the disease, three main histopathological alterations occur: cerebral atrophy, intracellular neurofibrillary tangles and amyloid plaques. Early loss of episodic memory in AD patients is closely associated with the progressive degeneration of the medial temporal lobe structures, with the transentorhinal cortex (TEC) being one of the first affected areas. This area is considered as a transitional zone between the entorhinal cortex (EC) and the temporal cortex. The main cytoarchitectonic feature of the TEC is that layers III and V merge and sweep obliquely to invade layer II of the EC. In this study, we performed a three-dimensional ultrastructural analysis of the neuropil from layer II of the TEC, using human brain tissue from 5 patients with AD and from 5 subjects with no apparent neurological diseases. We used an instrument that combines a high-resolution field-emission SEM column with a focused gallium ion beam (FIB), which mills the sample surface on a nanometer scale. The sequential and automated use of FIB milling and SEM imaging allows us to obtain large images stacks that represent a three-dimensional sample. Customized analysis software was used for the reconstruction of synapses, which allowed their number, morphology and spatial distribution to be calculated. Our preliminary results show that the total number of synapses per volume in layer II of the TEC in AD patients was significantly lower than in non-demented subjects. We have not found differences in the morphology of the synapses in AD patients compared with non-demented subjects. In addition, the spatial organization of synapses in the neuropil of layer II of the TEC is random, regardless of the subject group.

POSTER 84

Principal Investigator: De Felipe Oroquieta Javier (CIBERNED, Madrid)

Title: **Synaptic circuitry of hippocampal ca1 region in Alzheimer's Disease: an electron microscopy study**

Authors: Montero-Crespo M; Domínguez-Álvaro M; De Felipe J; Alonso-Nanclares L; Blázquez-Llorca L.

Abstract: Alzheimer's disease (AD) is the most common form of dementia, characterized by a persistent and progressive impairment of the upper cerebral functions. AD is typically associated with extracellular deposits of amyloid β (Aβ) and accumulation of abnormally phosphorylated tau protein inside neurons, leading to the formation of neuropil threads and intraneuronal neurofibrillary tangles (NFT), which are associated with neuronal degeneration and dendritic spine and synapse loss. Synaptic loss and failure are early pathological events that constitute the major neurobiological basis of cognitive dysfunction in AD and are already detectable in patients with mild cognitive impairment (MCI), a prodromal state of AD. Pyramidal cells from the hippocampus seem to be especially vulnerable and are affected early in AD. Taking this into account, the current study analyzes at the ultrastructural level the neuropil of the medial superficial CA1 pyramidal region of the human hippocampus from AD autopsies at different disease stages. Human brain samples with short postmortem periods (less tan 3h) and Focused Ion Beam milling/Scanning Electron Microscopy (FIB/SEM) were used to reveal and quantify possible alterations in synaptic connectivity. FIB/SEM technology has proved very useful for obtaining images with a quality and resolution similar to those acquired by transmission electron microscopy, but also has the great advantage of permitting the rapid and automatic serial section of large tissue volumes. It enables the three-dimensional analysis of different elements in the sample. The synaptic density, morphometric characteristics and spatial distribution of the synapses were examined with a specific software tool. Preliminary results show differences in some of the variables in the autopsies of patients suffering from AD. Therefore, elucidation of the changes that affect synapses is crucial for better understanding the pathogenic mechanisms underlying AD