Microglia were immunolabeled for Iba1 to study morphology under basal conditions and after exposure either to a pro-inflammatory (lipopolysaccharide) or a phagocytic (synaptosomes) stimulus. While cortical microglia from male VPA animals showed a pro-inflammatory profile and an intrinsic resistance to phagocytic stimuli, hippocampal microglia from male VPA animals matched microglia from controls under basal condition and showed a preserved response to pro-inflammatory and phagocytic stimuli. In the case of microglia isolated from females, both cortical and hippocampal microglia from VPA rats evidenced morphological changes under basal conditions but both were able to respond to pro-inflammatory and phagocytic stimuli. To sum up, microglia from male and female VPA rats show sex-dependent changes which may contribute to sex-differences in ASD.

405. (256) **A DEFICITARY MODEL OF CDK5 DOES NOT IMPAIRS NEURONAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS**

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CDK5/P35 is a complex involved in neuronal homeostasis and development that was described as a critical player for neuronal survival. Besides, its deregulation is linked with neurodegenerative pathologies such as Alzheimer Disease and Parkinson Disease. For that reason, we generated a deficitary CDK5 genetic model in neurons derived from human pluripotent stem cells. For this purpose, we used CRISPR/Cas9 technology to generate human embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively) KO-CDK5 lines. CDK5 protein expression levels were analyzed by western blot in samples obtained from clones where indels caused by CRISPR/Cas9 editing were detected by DNA sequencing. We obtained CDK5-/- clones for H9 hESCs and FN2.1 hiPSCs lines and a CDK5+/- clone for H9 hESCs line. Then, neural stem cells (NSC) were derived from the CDK5 KO clones using a commercial neural induction medium and their phenotype was validated by immunofluorescence staining using antibodies that recognize specific lineage markers (SOX-1, SOX-2, NESTIN and PAX-6). Finally, NSC obtained from the heterozygous CDK5+/- KO H9 hESCs clone were differentiated into neurons using a 2D-based protocol and their phenotype was validated by immunofluorescence staining of neuronal specific markers (TUJ-1 and MAP2). In conclusion, we managed to obtain NSC-neurons from CDK5-/- and CDK5+/- clones, determining that CDK5 is not essential for NSC generation. Besides, neuronal differentiation was achieved for H9 CDK5+/- clone, indicating that the CDK5 deficiency does not impair the generation of NSC-derived neurons. This result allows us to account with a CDK5-deficient model to further study its participation in neuronal homeostasis dysfunctions.

406. (266) **ASTROCYTIC INSULIN SIGNALING AND INFLAMMATION IN EXPERIMENTAL ALZHEIMER'S DISEASE**

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Insulin resistance (IR) and chronic inflammation are associated with the development of cognitive disorders and neurodegenerative diseases such as Alzheimer's (AD). However, it is not clear whether there is a causal link between these factors, which one appears earlier in the pathology or if either one of them is triggered by the increasing circulating levels of Aβ or amyloid deposits in early AD. Our objective was to study the metabolic and inflammatory status of a model of AD, the PDAPP-J20 mouse at the age of 8 months. We also treated a WT group with a high fat diet (HFD) as a positive control for IR. Our hypothesis was that in early stages of AD, the brain develops IR with astrocytes showing reactivity and impaired insulin signaling. Final body weight, glycemia and insulinemia were not affected by genotype or HFD. The open-field test showed an anxious-like behavior in transgenic and in HFD-fed mice. Insulin signaling measured by pAkt/Akt ratio was decreased in the hippocampus of AD mice ($p<0.05$) but not in the hypothalamus or the liver. Pancreatic IL1β and COX2 levels were unchanged. Insulin receptor puncta colocalizing with GFAP+ cells in the hippocampus by fluorescent immunolabeling showed a decreasing tendency in transgenic animals while astrocytic reactivity markers GFAP and S100b were increased ($p<0.05$). Finally, we evaluated the effect of fibrillar Aβ or palmitate on C6 astrocytes in vitro. Astrocytes exposed to Aβ showed increased nuclear translocation of NFkB and decreased AKT phosphorylation ($p<0.05$), suggesting inflammatory activation and impaired insulin signaling, respectively. Our results show that inflammation and insulin signaling impairment in the hippocampus are found in an early stage of experimental AD. The inflammatory context triggered by increased circulating Aβ or amyloid deposits in the brain could affect astrocytic insulin receptors, hence decreasing insulin signaling and affecting their neuroprotective capacity.

407. (267) **DIETARY RESTRICTION AS A FASTING MIMETIC IN AGED MICE. METABOLIC, COGNITIVE, AND NEUROINFLAMMATORY EVALUATION.**

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Aging is a physiological process that involves cognitive decline, decreased autophagic flux, and increased oxidative stress. Dietary restriction is a multitarget strategy that has been linked to several benefits, inducing autophagy flux, decreasing oxidative stress and inflammation, and improving metabolism. These effects establish dietary restriction as a possible approach to delay physiological aging and to prevent or treat aging-related diseases. In a previous work, we evaluated a protocol of periodic dietary restriction (PDR) in an animal model of familial Alzheimer's disease. Now, we have studied the effects of this strategy on aged female mice (16 month-old), evaluating metabolic, cognitive, and neuroinflammatory changes.
PDR involved 5 days of dietary restriction (DR) alternated with 9 days of ad libitum (AL) food intake for 7 weeks. During the DR period, mice ate 60% of their habitual intake.
Animals under PDR showed similar body weight and glycemia to AL mice. During DR periods, circulating ketone bodies increased (1WAnova-Sidak, basal vs DR $p<0.001$) suggesting a fasting-like effect. Additionally, we evaluated cognitive performance by the novel object location recognition test. No changes were observed between AL and DR animals, but both groups' performance was worse than that of 5 month-old mice, evidencing an age-related cognitive decline. We assayed S100b/GFAP by immunofluorescence in the hippocampus and analyzed morphological astrocytic parameters. S100b, an astrocytic pro-inflammatory marker, was diminished in DR mice (vs AL). However, GFAP immunoreactivity was unchanged. These preliminary results evidenced fasting-like effects in mice exposed to DR. Further, cognitive impairment in aged mice was corroborated, and a possible modulation of the pro-inflammatory S100b with DR. Future perspectives point to evaluating glial morphology in depth, and autophagy as a possible main mechanism for DR.

408. (268) **ADMINISTRATION OF ANASTRAZOLE, AN AROMATASE INHIBITOR, REDUCES THE PROTECTIVE EFFECTS OF TESTOSTERONE TREATMENT IN AN ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

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