P1-095 A NEW *IN VITRO* SCREENING LIAISON: DIRECT EFFECTS ON BETA-AMYLOID OLIGOMER FORMATION AND BENEFICIAL IMPACTS ON NEUROPROTECTION

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Background: Alzheimer's disease (AD) is one of the most devastating neurodegenerative diseases. Generation of amyloid-beta peptide (Aβ) species and their aggregation into highly neurotoxic oligomers and fibrills is one of the major hallmarks in AD. Early formation of intracellular Aβ aggregates is already accompanied with cognitive impairment in AD patients, far before plaques are formed. Interfering with Aβ aggregation is an inevitable strategy in the development of novel therapeutic approaches. The aim of this study was to provide a fast, quantitative and reliable in vitro screening assay capable of showing direct effects of compounds on Aβ oligomer formation and thus, a beneficial impact on cell viability. **Methods:** Additional to the Amorfix Aggregated Aβ Assay (A4), neuroprotection assays were used to characterize two in vitro screening models; primary chicken neurons and H4 neuroglioma cells overexpressing human APP K595N/M596L. **Results:** New developmental compounds Y and Z, when co-aggregated with Aβ1-42, were able to reduce A β oligomer formation in vitro as determined by A4 assay. These same compounds rescued Aβ induced toxicity in primary neurons. A second screening model the H4 neuroglioma cells overexpressing human APP K595N/M596L, comprising the Swedish double mutation, already formed Aβ oligomers in steady state conditions which could be efficiently cleared by the y-secretase inhibitor DAPT. **Conclusions:** Combining the highly sensitive A4 Assay with neuroprotection assays enables fast and quantitative in vitro screens for the development of promising drug candidates in Alzheimer's disease.

P1-096 THE ROLE OF FBXO2 IN APP TURNOVER AND PROCESSING

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Background: vvThe Amyloid Precursor Protein (APP) plays a critical role in the pathogenesis of Alzheimer's disease (AD) which now affects more than five million Americans. Recent studies have linked the ubiquitin-proteasome system (UPS) to the trafficking of APP and to the turnover of enzymes that cleave APP. The extent to which the UPS governs the amount, distribution, and processing of APP, however, remains uncertain. Fbxo2, a ubiquitin ligase substrate adaptor protein enriched in mammalian neurons, has recently been reported to affect the levels of beta-site APP cleaving enzyme 1 (BACE1) and thereby influence the production of the amyloid-beta (A b) cleavage product. Intriguingly, decreased levels of Fbxo2 have been reported in postmortem tissues of Alzheimer's patients, suggesting that this ubiquitin pathway protein could contribute to AD pathogenesis. **Methods:** Fbxo2 -/- mice were age-matched at postnatal day 4 and three, six, nine, and twelve months of age to Fbxo2 +/+ mice and examined using western blot, ELISA, and immunohistochemical techniques. Cultured hippocampal neurons and HEK cell-based models were similarly evaluated. **Results:** We find that APP is itself a substrate for Fbxo2. In the Fbxo2 knockout mouse, loss of Fbxo2 in vivo results in increased levels of endogenous murine APP. The distribution of APP within neurons is also altered in the absence of Fbxo2, with a greater amount of APP co-localizing with synaptic markers. Furthermore, the amount of A b 1-40, A b 1-42, and soluble alpha-secretase cleavage products secreted by hippocampal neurons are each doubled when Fbxo2 is absent. **Conclusions:** Taken together, these results suggest that the loss of Fbxo2 may play a role in the pathogenesis of AD. Future studies will examine the mechanisms regulating the ubiquitination of APP by Fbxo2 as well as the impact of decreased Fbxo2 levels in AD mouse models.

P1-097 PHYSIOLOGICAL LEVELS OF BETA-AMYLOID PROTEIN HELP MAINTAIN NORMAL GSK AND TAU BALANCE AND IMPROVE MEMORY

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Background: Amyloid beta protein (Aβ P) is well recognized as having a significant role in the pathogenesis of Alzheimer's disease (AD). The reason for the presence of Aβ P and its physiological role in non-disease state s is just beginning to come to light. We have previous shown that AβP injected into the hippocampus of normal CD-1 mice with normal memory improved memory at low doses and impaired memory at high doses. In addition, we demonstrated that AβP administered to hippocampal slices of CD-1 mice enhanced LTP. **Methods:** PC12 cells were obtained from Dr. Amy Harkins (St. Louis University School of Medicine, Department of Pharmacological and Physiological Sciences) and cultured in our laboratory. After 4-5 days cells were treated with AβP pM for 10 minutes and tested for serine glycogen synthase kinase and Tau via western blot procedure. In a second set of experiments, we tested the effects of AβP injected into the hippocampus of CD-1 mice. Mice were trained in step-down passive avoidance. Immediately after training, mice received a bilateral hippocampal injection of AβP 8.7ng/µl. Retention was tested 24 hours after training. **Results:** The result indicate that AβP administered to PC12 cells increased the percentage of phosphorylated GSK/GSK at 10 pM. and decreased the value at 200nM.The percentage of phosphorylated Tau/Tau was decreased by both doses of beta-amyloid. In step-down passive avoidance 8.7ng improved retention. The mice that received AβP had a retention score (Latency Retention - Acquisition) of 67.00 $\pm$ 21.88 and the vehicle mice had a retention score of 27.87 $\pm$ 12.00 ($p<0.05$). **Conclusions:** The current findings are further proof to that AβP at physiological levels is essential in the brain for normal cognitive function by interacting with pGSK to GSK and to keep the balance of pTau to Tau. In addition, we provide further evidence that small increases in AβP improve memory.

P1-098 DISSECTING THE FUNCTION OF THE AMYLOID PRECURSOR PROTEIN

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Background: The amyloid precursor protein (APP) is genetically linked to Alzheimer's disease, but the molecular process that APP controls remains elusive. Our previous work has shown that APP binds to APP-BP1 (BP1), a major activator of ubiquitination. BP1 is the activating enzyme of the small ubiquitin-like protein nedd8, which covalently modifies and activates Cullin ubiquitin ligases. One of the Cullins, Cul5, is implicated in downregulating inflammatory response using SOCS proteins as substrate-recognition subunits. **Methods:** We examined the effect of APP on Cul5in primary neurons andin APP knockout animals by western blotting and immunostaining. **Results:** We found that the steady-state levels of Cul5 was very low in primary neurons, but was significantly increased when APP wassuppressed by shRNAs.Similarly, Cul5 was increased in APP knockout neurons compared with the wild type controls in vitro. Analyses of Cul5- stained brain tissues also revealed that Cul5 was signigicantly elevated in APP knockoutneurons compared with the wild type littermate controls.Cul5 was not induced by IL-1b in neurons, but IL-1b induced the expression of SOCS3 in a dose-dependent manner. Furthermore, Cul5 became stabilized only when the ubiquitin-activating enzyme or the nedd8-activating enzyme were inhibited. **Conclusions:** We conclude that a physiological function of APP is tomaintain Cul5 ligase activity. Stabilized Cul5 is likely the inactive form of Cul5 and accordingly, without APP, neurons would fail to mount a proper response to signaling molecules such as IL-1b. These findings indicate that APP dysfunction is involved in Alzheimer's disease pathogenesis.