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**Background:** Alzheimer's disease (AD) is characterized by cognitive decline and hallmark neuropathologies, including β-amyloid (Aβ). Therapeutic strategies for AD focus on reducing the production or deposition of Aβ. Canines develop Aβ neuropathology and cognitive decline with age similar to AD patients, making them a useful model for testing potential therapies. Immunization with Aβ1-42 (IMM)in aged canines significantly decreases brain Aβ and maintains executive function. However, behavioral enrichment (BEH) improves cognition without reducing brain Aβ. We hypothesized that IMM combined with BEH would provide larger cognitive benefits and further reduce neuropathology, as compared to controls or individual IMM and BEH treatments alone. **Methods:** Aged beagles (10.5-13.6 y) were placed into groups: control (Alum adjuvant only), fibrillar Aβ1-42 + Alum vaccine, BEH with Alum, and combination treatment (IMM+BEH). Animals were treated for 18 months. Cognition was measured throughout the study using various learning and memory tasks. Serum IgG antibody titers, cerebral spinal fluid (CSF) Aβ levels, and insoluble brain Aβ (formic acid extracted) were measured by ELISA. Aβ plaque load (6E10, anti-Aβ 1-42, Pyro Glu3), CAA (congo red), and microhemorrhages (prussian blue) were measured by immunohistochemistry. **Results:** Anti-Aβ1-42 IgG responses in IMM animals increased significantly and were maintained. BEH significantly increased CSF Aβ1-40, while no systematic effects were seen on Aβ1-42 or total Aβ. IMM significantly reduced both insoluble Aβ1-40 and 1-42, as well as all forms of measured plaque load. An overall reduction in 6E10, and Aβ 1-42 plaque load due to BEH was also seen. A significant additive affect from BEH and IMM was seen in clearance of Aβ 1-42 plaque load. **Conclusions:** IMM successfully induced an immune response in treated animals and was maintained. IMM significantly reduced all forms of Aβ pathology. Additionally, BEH increased CSF Aβ 1-40 and reduced 6E10 and Aβ 1-42 plaque loads. A significant additive effect from BEH and IMM was seen in clearance of Aβ 1-42 plaque load. While changes in cognition, CAA and microhemorrhages remain to be analyzed, it is expected that levels of CAA and microhemorrhages will be lower in IMM animals, and IMM+BEH will show greatest cognitive benefits.

## P1-065 CROCUS SATIVUS STIGMA EXTRACT IMPROVES AMYLOID-B DEGRADATION IN MONOCYTES FROM ALZHEIMER'S DISEASE PATIENTS

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**Background:** Alzheimer's Disease (AD) is the most common form of dementia among people over the age of 65, accounting for 50-60% of all cases. The present study investigates the possible reversal effects of saffron in the reduced amyloid- b degradation by monocytes from AD patients. **Methods:** Crocus sativus L. was collected from Umbria (Central Italy) and kindly provided by the Associazione dello Zafferano di Cascia - Zafferano Purissimo dell'Umbria. Freshly purified monocytes before and after in vitro treatment with Crocus sativus extract (16 m M) were incubated for 24 h with 5 m g/ml Amyloid- b(HiLyte Fluor™ 488 - labeled, 60479-01, AnaSpec Inc.) in the culture medium in order to test A b42 phagocytosis and degradation by monocytes of 10 healthy subjects and 11 probable AD patients. Cells were harvested and washed twice with ice-cold PBS and analyzed by flow cytometry (Beckman Coulter EPICS XL). **Results:** Monocytes from AD subjects pre-treated with saffron extract showed an improved ability to degrade Aβ42. Although with a percentage similar to untreated AD monocytes, a reduced fluorescence was observed ($p<0.05$ vs. untreated AD cells), indicating a more rapid A b 42 degradation compared to untreated AD monocytes. **Conclusions:** E ven though more studies are needed to identify the active molecules, or their combinations, responsible for the enhanced A b42 degradation, these data suggest a potential beneficial effect of saffron on AD patients in terms of A b42 clearance.

## P1-066 TREATMENT WITH SP1 INHIBITING DRUGS MODULATES APP AND BACE1 LEVELS IN HUMAN CELLS: IMPLICATION IN TESTING A NOVEL TARGET FOR ALZHEIMER'S DISEASE

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**Background:** Alzheimer's disease (AD) is believed to result from misregulation of amyloid-β (Aβ) production, which forms the plaques in AD brains. The rate-limiting step in the production of Aβ is the processing of amyloid-β precursor protein (APP) by β-site APP-cleaving enzyme (BACE1). The transcription factor specificity protein 1 (SP1) coactivates the transcription of BACE1 and APP. We used human glioblastoma cells U373 along with human neurosphere cultures to demonstrate activity of SP1-mediated regulation of APP with Mithramycin A, a selective inhibitor of SP1, and Tolfenamic acid, which induces the degradation of SP1. **Methods:** Neurospheres (NSP) were cultured in Neurocult Basal Media plus Differentiation Supplement (Stem Cell Technologies). U373 were obtained from ATCC. Cells were cultured, transfected, and Western blot analysis was performed as previously described (Long et al., JBC-2014). Mithramycin A (Santa Cruz) and Tolfenamic acid (Sigma Aldrich) were prepared in 1 μ M and 5 μ M doses. After 72-hour treatment or transfection, lysates were collected. Cell viability was assessed using Cell-Titer Glo (CTG) assay (Promega). **Results:** Western blot analysis reveals a significant decrease in the expression of APP in U373 and NSP treated with Mithramycin A. NSP treated with Mithramycin A also exhibit a decrease in BACE1 expression. Treatment with Tolfenamic acid, however, does not significantly decrease APP or BACE1 expression in either cell model. APP siRNA effectively knocks down APP protein expression in U373 and in NSP. BACE1 siRNA and SP1 siRNA did not significantly affect APP levels in either cell model, suggesting cell specific effects. In U373, BACE1 protein expression is significantly decreased with BACE1 siRNA transfection. **Conclusions:** We show that expression of APP is decreased after treatment with SP1 inhibitor, Mithramycin A in both U373 and cells derived from human neurospheres. However, APP expression is not affected by treatment with Tolfenamic acid, perhaps due to the differences in the mechanisms between these SP1 inhibiting drugs. We also show that transfection with siRNAs can effectively change the expression of APP and BACE1 in human cells. It is important to discover whether drugs or small RNAs targeting this transcription factor could be used to effectively decrease amyloid load and possibly the symptoms of AD in patients.

## P1-067 OBESITY-INDUCED INSULIN RESISTANCE INCREASES APP T668 PHOSPHORYLATION AND IMPAIRS COGNITIVE FUNCTION

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**Background:** Diet-induced obesity is a risk factor for Alzheimer's disease (AD). Insulin resistance (IR) is a major feature of the metabolic syndrome including obesity and type 2 diabetes. Impaired insulin/insulin-like growth factor (IGF-I) signaling cascades due to IR may provide a link between obesity, diabetes, cognitive impairment and AD. In addition to the well-known pathological features of increased tau phosphorylation and A b accumulation in AD, recent studies also highlight the critical role of

phosphorylation of amyloid precursor protein (APP). When phosphorylated at threonine 668 (T668), APP undergoes conformational changes affecting its intracellular sorting and trafficking, which in turns impact proteolytic cleavage and increases A b production. The role of APP T668 phosphorylation and obesity in AD are not well understood and indicate a critical need to understand the mechanism(s) linking obesity and cognitive decline. **Methods:** Obesity is induced in C57Bl/6 mice using a high fat diet (54% kCal from fat) for 24 wk. APP and tau phosphorylation is examined from cortex lysates. Cortical neurons are prepared from E15 rat embryo and cultured in vitro for 7 days before insulin and/or IGF-I treatment. **Results:** Obese mice displayed significant cognitive impairment at 24 wk in parallel with the increased tau and T668-APP phosphorylation in the cortex. We previously reported that embryonic cortical neurons (eCN) develop neuronal IR with decreased insulin and IGF-I signaling following chronic insulin treatment. IGF-I treatment of eCN decreased T668-APP phosphorylation. Insulin also decreased APP phosphorylation but the effect was much weaker compared to IGF-I. Chronic treatment of eCN with insulin increased basal T668-APP phosphorylation. IGF-I was still able to reduce T668 phosphorylation after chronic insulin treatment; insulin itself was unable to reduce APP phosphorylation. These effect was reversed with the simultaneous treatment of chronic insulin with PI3-K inhibitor, suggesting chronic hyperactivation of Akt is responsible for IR-induced APP phosphorylation. **Conclusions:** Our results suggest IR-induced increases in T668 phosphorylation of APP as a possible link between obesity and cognitive impairment. Furthermore our data reveal a potential and beneficial effect of IGF-I signaling as a therapeutic target. This work is supported by the Program for Neurology Research and Discovery (www.med.umich.edu/PNRD).

## P1-068 A PHYSIOLOGICAL ROLE FOR AMYLOID BETA IN CYCLIC AMP-STIMULATED LONG TERM POTENTIATION

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**Background:** Cyclic adenosine monophosphate (cAMP) regulates long-term potentiation (LTP) and ameliorates memory in healthy and diseased brain. Increasing evidence also shows that, under physiological conditions, low concentrations of amyloid b (A b) are necessary for LTP expression and memory formation. Based on these evidences, we tested the hypothesis of a functional correlation between cAMP, A b and LTP, in an attempt to reveal novel molecular mechanisms for memory formation. **Methods:** In neuronal cultured cells and rat hippocampal slices, expression of the A b precursor protein (APP) was measured by RT-PCR and immunoblotting, whereas A b 42 was analyzed using specific ELISA. Electrophysiological LTP recordings were performed in hippocampal slices from wild-type and APP knockout mice. **Results:** Our study shows, for the first time, that cAMP enhances LTP by stimulating the synthesis of APP and, in turn, the production of A b. In particular, our results indicate that PKA but not EPAC is involved in the cAMP-induced increase of APP and A b 42. Moreover, we demonstrate that cAMP requires translation, but not transcription, in order to increase APP and A b levels. Finally, we show that the reinforcing effects of cAMP on LTP are abolished in APP knockout mice, where A b cannot be produced, and are prevented in wild-type animals when the extracellular peptide is depleted by anti-A b antibodies. **Conclusions:** The present data demonstrate that endogenous cAMP requires APP and A b to boost hippocampal LTP. Collectively, our study has revealed a novel cAMP/PKA/APP/A b molecular pathway through which the second messenger positively influences the cellular mechanisms of memory formation and adds further evidence for a physiological role of A b. Research supported by grants from Alzheimer's Association (NIRG-07-59597 to D.P. and IIRG-11-208306 to O.B.) and Fondazione CARIGE (to M.A.P.).

## P1-069 A PRESENILIN 1 MUTATION ALTERS APP LOCALIZATION IN HUMAN NEURONS

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**Background:** Presenilin 1 (PS1) is the catalytic core of the γ-secretase complex that cleaves the Amyloid Precursor Protein (APP) and other type 1 transmembrane proteins. Mutations in PS1 are the most common cause of early onset Alzheimer's disease (AD). Despite extensive research into the mechanisms by which PS1 mutations cause AD, there is still uncertainty in the precise mechanisms by which PS1 mutations initiate disease. **Methods:** We utilized isogenic induced pluripotent stem cell (iPSC) derived neurons that harbor the PS1 Δe9 mutation to investigate neuronal phenotypes caused by mutant PS1. Additionally, we used quantitative immunofluorescence to measure APP localization and APP colocalization with endocytic markers. **Results:** We report that the PS1 Δe9 mutation alters localization of APP such that there is decreased APP in axons and increased APP in the cell body. Additionally we found that there is decreased colocalization of APP with the early enodcytic marker Rab5 in axons. **Conclusions:** Our results suggest that abnormal APP localization may be an early event in progression of familial AD.

## P1-070 DEVELOPMENT OF AN IMPROVED IMMUNOASSAY FOR DETECTION OF SORLA IN CELLS AND BIOLOGICAL SAMPLES

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**Background:** SorLA (Sorting - related receptor with A- type repeats) is a 250 kDa type I transmembrane protein, belonging to the VPS10P (vacuolar protein sorting 10 protein) family of neuronal receptors. It is implicated in the development of AD (Alzheimer's Disease), atherosclerosis, diabetic retinopathy, and acute leukemia. Despite the overwhelming evidence regarding the role of sorLA in various diseases, there has been a lack of technologies which can precisely quantitate the levels of sorLA in various complex biological matrices. The methods are either qualitative like immunohistochemistry, or traditional sandwich ELISA assays which are time consuming and less sensitive. Hence, the purpose of the present study is to develop a new assay called AlphaLISA which is fast and very sensitive, to measure sorLA in extremely small volumes of cells and biological samples. **Methods:** The AlphaLISA is a homogenous bead based assay using donor and acceptor beads. The donor bead is coated with streptavidin that captures a biotinylated antibody while the acceptor bead is coated with analyte-specific antibody. When brought into proximity through binding to the analyte and excited by laser at 680 nm, the donor bead releases singlet oxygen which triggers a series of chemical reactions in the acceptor beads causing a sharp peak of light emission at 615 nm. A series of experiments were designed to optimize the assay by conjugation of the beads to various anti-sorLA antibodies, cross titrations of the antibodies, spike and recovery experiments to check matrix interference, signal to noise ratio determined for the counts, and comparison of our novel immunoassay in terms of sensitivity with existing methods. **Results:** Our results show that as compared to traditional methods, AlphaLISA is a sensitive and rapid assay, which can be automated suitably for determination of sorLA in large sample batches. It also shows high recovery and signal to noise ratio. **Conclusions:** The results support the development of an improved method for measuring sorLA quantitatively, which could further prove as an important tool in investigation and establisment of sorLA as a potential biomarker in AD.