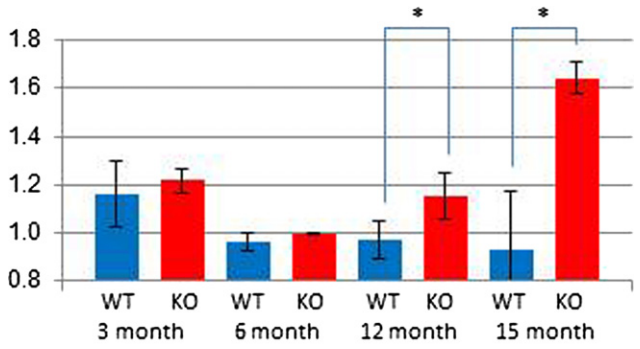

Figure 4. Altered NPY levels in hippocampus tissue lysates analyzed by dot blot. NPY levels are increased in 12 and 15 month old ZnT3-/- hippocampus compared to wild type hippocampus. (n=4 in 3 and 15 month old, n=3 in 6 month old and n=5 in 12 month old. *$P \leq 0.05$).

month-old subjects to determine the presence of seizures in ZnT3-/- and age-matched control mice. **Results:** We found an age-dependent decrease in calbindin levels in ZnT3-/- mouse hippocampus compared to age-matched control mice. There was no difference at three months of age, a 12% decrease at six months of age, an 18% decrease at twelve months of age, and a 16% decrease at 15 months of age. Analysis of neuropeptide Y levels indicates an age-dependent increase in ZnT3-/- mouse hippocampus compared to age-matched control mice. There was no difference at three and six months of age, a 16% increase at twelve months of age and a 43% increase at 15 months of age. **Conclusions:** The finding of alterations in protein levels consistent with seizure activity in ZnT3-/- mice indicates that sequestration of zinc may result in reduced excitatory modulation by zinc leading to hyperexcitability and increased seizure activity. This provides new insight into the mechanisms responsible for the seizure activity common in AD patients and potential targets for therapeutic interventions to ameliorate AD-related seizures.

P4-034 ALZHEIMER'S DISEASE HUMAN BRAIN BETA-AMYLOID-CONTAINING EXTRACTS INHIBIT HIPPOCAMPAL LONG-TERM POTENTIATION IN RATS *IN VIVO*: RELATIONSHIP BETWEEN SOLUBLE AND INSOLUBLE PREPARATIONS

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Background: Previously, we showed that water-soluble extracts from Alzheimer's disease (AD) human brain contain aggregates of amyloid-beta protein (Aβ) and inhibit long-term potentiation (LTP), a form of synaptic plasticity believed to be necessary for successful memory function (Barry et al., 2011; Freir et al., 2011). Here, we tried to evaluate the effects of insoluble, plaque core-containing preparations from AD brain. **Methods:** In vivo experiments were carried out on urethane-anaesthetized rats under the approval of Trinity College Dublin's ethical review committee and of the Department of Health and Children, Ireland. Single pathway recordings were made from the CA1 stratum radiatum. The stimulation protocol for inducing LTP consisted of 10 trains of 20 stimuli at 200 Hz, intertrain interval 2 s. Human brain extracts were injected i.c.v. into the right lateral ventricle. Post mortem human brain tissue was obtained and used in accordance with the Trinity College Dublin Health Sciences Faculty Ethics Committee guidelines. Statistical comparisons were made using Student's t test. **Results:** In control, vehicle-injected animals conditioning stimulation induced significant LTP. Aβ-containing tris-buffered saline (TBS) extracts from AD brains significantly reduced LTP. Animals injected with washed TBS-insoluble pellets from AD brain produced significant LTP indistinguishable from control. Although co-injection of insoluble AD pellets did not significantly affect inhibition of LTP by Aβ-containing soluble extract, the difference between control and co-injected groups was also not significant. A potential role of insoluble Aβ in plaque cores was also explored using synthetic Abeta fibrils. Similar to insoluble AD brain extracts, synthetic Aβ fibrils did not significantly affect control LTP. Moreover, the magnitude of potentiation in animals co-injected with soluble and insoluble Aβ was not significantly different from vehicle controls. **Conclusions:** Insoluble Aβ in plaque cores appears not to affect LTP in vivo. Whether or not it can sequester synaptic plasticity-disrupting soluble Aβ requires further research.

P4-035 A NEW *IN VITRO* FUNCTIONAL ASSAY ON PRIMARY CORTICAL CULTURES SHOWS THAT PPAR-GAMMA AGONISTS ARE NEURO-PROTECTIVE AGAINST MPP+ BUT NOT BETA-AMYLOID-INDUCED SYNAPTOTOXICITY

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Background: Most in vitro assays for Alzheimer's and Parkinson's Disease (AD and PD) are based on high throughput detection of cellular death or on sophisticated but labor-intensive analysis of cellular functions such as synaptic plasticity. We report here the development of a new assay that monitors integrated cellular function to test neuro-protective drug candidates in a high throughput mode. **Methods:** Primary cortical cultures from embryonic E16 mice were plated in 384-well plates. At day 7, cells were treated with oligomeric Aβ1-42 or the PD-symptom inducing neurotoxin MPP+, either in presence or absence of potentially protective compounds. 24h later, compounds, Aβ and MPP+ were washed off, cells were loaded with fluo-4, and synchronized network activity was measured in a fluorescence plate reader (FLIPR). Changes in oscillation frequency and amplitude, acute responses to glutamate or ATP, and released adenylate kinase activity were used to assess network integrity, cellular function and cell viability. **Results:** Application of oligomeric Aβ1-42 but not Aβ1-40 reduced the amplitude but not frequency of network oscillations with an IC50 value in low μM range. MPP+ also reduced oscillation amplitudes with an IC50 of 6 μM. At 10 μM, MPP+ did no impact cell viability as measured by a standard non-invasive toxicity assay. The PPARgamma agonist rosiglitazone was able to concentration-dependently prevent the effect of MPP+ but not of Aβ1-42 on network activity. **Conclusions:** A functional, phenotypic assay suitable for high throughput screening was developed based on synchronized network activity of primary cortical cultures. We observed a robust reduction of network activity by Aβ1-42 oligomers and the neurotoxin MPP+ at concentrations that did not yet affect cell viability. Corroborating earlier observations in vivo, stimulation of PPARgamma could protect neurons against MPP+ effects in this assay. Our finding that rosiglitazone did not protect against Aβ1-42 effects suggests that MPP+ and Aβ1-42 affect different pathways that lead to impairment of neuronal activity. In conclusion, this assay can facilitate drug discovery and development in neurodegenerative diseases such as AD and PD.

P4-036 AMYLOID PRECURSOR PROTEIN REGULATES NEURONAL CHOLESTEROL TURNOVER NEEDED FOR SYNAPTIC ACTIVITY

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Background: Processing of Amyloid Precursor Protein (APP) by b- and g-secretase activities produces b-amyloid peptide, which accumulates in extracellular senile plaques present in brain of patients with Alzheimer disease (AD). Although APP processing by secretase activities has been extensively studied, the function of the protein remains largely

unknown. Recently, genome wide association studies on AD confirmed that ApoE4 is a major risk factor and identified new susceptibility loci, which further support the hypothesis that perturbation of lipids metabolism favours progression of AD. Therefore, we have studied the influence of human APP (hAPP) expression on neuronal or astrocytic cholesterol synthesis. **Methods:** hAPP was expressed in rat cultured neurons and astrocytes using recombinant adenoviruses. Biosynthesis of cholesterol was measured following 14 C acetate cellular incorporation. mRNAs were quantified by qRT-PCR. Sterol regulatory element binding proteins (SREBP) and APP were quantified in cellular or brain extracts by western blotting, their subcellular localisation was analyzed by immunocytochemistry and their interaction by co-immunoprecipitation. Synaptic activity was measured in cultured neurons by c alcium oscillations using Fura-2AM, and in vivo by induction of long-term potentiation (LTP). **Results:** Expression of hAPP in primary cultures of rat cortical neurons decreased HMG-CoA reductase-mediated cholesterol biosynthesis, as well as SREBP-1 and -2 mRNA levels. In neurons, APP and SREBP1 co-immunoprecipitated and co-localized in the Golgi. This interaction prevented Site-2 protease-mediated release of mature SREBP, leading to inhibition of transcription of its target genes. In cultured astrocytes, APP and SREBP1 did not interact nor did APP affect cholesterol biosynthesis. Neuronal expression of hAPP also decreased cholesterol 24-hydroxylase mRNA levels and consequently cholesterol hydroxylation, leading to inhibition of cholesterol turnover. Geranylgeraniol, generated in the mevalonate pathway upon changes in cholesterol turnover, rescued defects in synaptic activity and LTP in hAPP expressing neurons and 5xFAD transgenic mice, respectively. **Conclusions:** T hese results argue for a major role of APP in the control of neuronal cholesterol turnover needed for synaptic activity both in vitro and in vivo.

P4-037 ISCHEMIA AS A TRIGGER FOR RAGE-DEPENDENT SYNAPTIC DYSFUNCTION IN AN AMYLOID-ENRICHED ENVIRONMENT

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Background: Alzheimer's disease (AD) is increased by vascular pathologies inducing brain hypoperfusion and hypoxia. However, a relationship between ischemia and AD has not been established. Recent understanding has confirmed that Receptor for Advanced Glycation Endproducts (RAGE) plays a role in the pathogenesis of AD. RAGE functions as a signal-transducing receptor for beta-amyloid (Aβ) leading to synaptic dysfunction and cognitive impairment. Our hypothesis is that ischemia facilitates brain impairment induced by A β signaling through RAGE. **Methods:** We evaluated the effect of acute transient ischemia on fEPSP recorded in acute slices containing enthorinal cortex (EC), a brain region primarily affected in AD. EC slices were exposed to oxygen-glucose deprivation (OGD) for a short period (10 min), a well known model of in vitro ischemia **Results:** OGD induced a fast depression of fEPSPs that partially recovered after re-introduction of oxygenated ACSF Absence of RAGE in EC slices from RAGE Knock-out mice resulted in a faster and complete recover of fEPSPs amplitude following OGD. The same protective effect was achieved in EC slices from Transgenic mice overexpressing a dominant negative form of RAGE selectively in microglia (DNMSR) but not in slices from mice with defective RAGE targeted to neurons. To test whether ischemia-induced synaptic dysfunction is increased in A β enriched environment, OGD effect on neuronal impairment was investigated in EC slices perfused with A β peptide or in slices from Tg mAPP mice. We found that OGD effects were enhanced in A β enriched environment and that RAGE inhibition protected from synaptic impairment induced by transient ischemia in Tg mAPP slices or in the presence of A β. In addition, we found that A β/RAGE interaction under ischemic condition activated an inflammatory pathway that is driven by RAGE expressed in microglia and involves the release of IL-1 β and phosphorylation of stress-related kinases (p38 MAPK and JNK) **Conclusions:** These results suggest that ischemia may function as a trigger for neuronal perturbation induced by progressive accumulation of Aβ and that RAGE could be an important factor in accelerating synaptic dysfunction in vascular pathology and AD.

P4-038 TRANSCRIPTIONAL SUPPRESSION OF AGRIN PROMOTES ALZHEIMER'S-LIKE SYNAPTIC AND NEUROVASCULAR PATHOLOGY IN PRIMARY NEURONS IN CULTURE AND MOUSE BRAIN

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Background: Alzheimer's disease (AD) is characterized by senile plaques, neurofibrillary tangles (NFTs), neuronal death, and synaptic and neurovascular dysfunction. Both synaptic and neurovascular dysfunction occurs early in AD, and has been shown to strongly correlate with cognitive impairment and disease progression. Agrin is a heparansulfate proteoglycan that is associated with neurite outgrowth and synaptogenesis, basement membranes of the blood-brain barrier (BBB), and AD plaques. In AD, the BBB is compromised, resulting in altered permeability and active transport from the blood to the brain. Importantly, current studies suggest that loss of agrin in the basement membrane of cerebral endothelial cells may interrupt BBB integrity. The mechanism of how agrin influences BBB maintenance, however, is not yet understood. Egr-1 is a transcription factor that is a critical mediator of vascular pathology, is increased in AD, and is involved in regulating several implicated genes. **Methods:** We primarily used lentiviral infection of primary neurons, mouse brain homogenate, Western blotting, electrophysiology, and relative standard curve SYBR green real-time PCR. **Results:** Interestingly, we found that agrin levels are significantly increased in a step-up manner in Egr-1+/- and Egr-1-/- mice compared to control, leading us to investigate a role of Egr-1 in the regulation of agrin. Using the rVista program, we have located five putative Egr-1 binding sites on the agrin promoter. Lentiviral overexpression of Egr-1 in primary neurons in culture causes a decrease in synaptic marker protein synaptophysin and in glutamate-mediated postsynaptic events. In these same cells, there was a 1.8 and 1.5 fold decrease, respectively, in agrin protein levels compared to controls. In Egr-1 infected neurons with a 1.8 fold and 5.5 fold increase in Egr-1 mRNA compared to control, there was a 14.4% and 44% decrease in agrin mRNA, respectively. **Conclusions:** Our data indicate that Egr-1 regulates transcription of agrin in the brain and suggests that increased Egr-1 levels in AD brain, by reduction of agrin, may cause synaptic loss and disruption of BBB integrity and/or maintenance. This provides the potential for a novel link between two very important and complex pathologies in AD, allowing for promising new research and ideas in the realms of AD therapeutics.

P4-040 PHOSPHORYLATION OF COLLAPSIN RESPONSE MEDIATOR PROTEIN-2 AND AXONAL DEGENERATION IN TRANSGENIC MICE EXPRESSING A FAMILIAL ALZHEIMER'S DISEASE MUTANT OF APP AND TAU-TUBULIN KINASE 1

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Background: Tau-tubulin kinase-1 (TTBK1) is a brain-specific protein kinase involved in tau phosphorylation at AD-related sites and up-regulated in AD brain. Recent large independent studies have identified significant association of two single nucleotide polymorphisms (SNPs) in the TTBK1 gene with late-onset AD (LOAD) in Spanish and Han Chinese populations. However its role in AD pathogenesis is poorly understood. We hypothesize that TTBK1 regulate axonal growth via phosphorylation of microtubule-associated molecules, such as tau and