degeneration of dopaminergic neurons in the substantia nigra and in the locus coeruleus, the same neurons that are lost in Parkinson's disease (PD). **Methods:** Human dopaminergic SH-SY5Y neuroblastoma cells have been stably transfected with wild-type (WT) Parkin and the Parkin ARJP mutants G328E and R42P. Co-immunoprecipitation has been used to investigate if Parkin interacts with PLC-γ1 followed by In vitro ubiquitin conjugation assay in order to examine if Parkin can ubiquitylate PLC-γ1. PLC activity was measured by Phosphoinositide hydrolysis assay. Intracellular Ca^{2+} measurements have been done by using the calcium indicator Fluo-3. Cell viability was determined by the MTT assay. **Results:** We show that Parkin is associated with Phospholipase C-γ1 (PLC-γ1) and that this association is impaired in ARJP Parkin mutants, affecting PLC-γ1 ubiquitination. Increased steady state levels of PLC-γ1 were noted in ARJP Parkin mutant cells, and in Parkin knockout mouse brain. Parkin mutants have enhanced basal phosphoinositide hydrolysis and intracellular Ca^{2+} levels ($[Ca^{2+}]_i$). Neomycin (PLC inhibitor) and dantrolene (Ryanodine receptor inhibitor) decreased $[Ca^{2+}]_i$ in Parkin mutants to those seen in WT Parkin cells, suggesting that differences were a consequence of altered PLC activity. Also, the protection of WT Parkin against 6-hydroxydopamine (6OHDA) toxicity could be mimicked in ARJP mutants by the addition of dantrolene, implying that affecting Ca^{2+} release from ryanodine-sensitive stores is sufficient to protect neurons from toxicity. **Conclusions:** Our findings indicate that PLC-γ1 is a substrate for Parkin. We suggest that loss of function in Parkin mutations leads to PLC-γ1 accumulation and to a disruption in downstream signalling that makes cells more vulnerable to neurotoxins such as 6OHDA.

P3-404 TRIPLE-TRANSGENIC ALZHEIMER'S DISEASE MICE EXHIBIT MARKED ABNORMALITIES IN BRAIN MYELINATION PATTERNS PRIOR TO APPEARANCE OF AMYLOID AND TAU PATHOLOGY

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to memory and cognitive impairment in afflicted individuals. Pathologically, AD is characterized by the temporal and spatial progression of amyloid plaques from deposition of the fibrillogenic Aβ peptide, neurofibrillary tangles from hyperphosphorylation of the microtubule stabilizing protein, tau, decreased synaptic density, and eventual neuronal loss. AD has not been widely considered a disease of white matter, but more recent evidence suggests the existence of abnormalities in myelination patterns and myelin attrition in AD-afflicted human brains. **Methods:** The myelination status of triple-transgenic AD (3xTg-AD) mice and non-transgenic mice at various ages was systematically examined using immunohistochemical, biochemical, and ultrastructural analyses. **Results:** Herein, we demonstrate that 3xTg-AD mice, which harbor the human amyloid precursor protein Swedish mutant transgene, presenilin knock-in mutation, and tau P301L mutant transgene, exhibit significant alterations in overall myelination patterns and oligodendrocyte marker expression profiles at time points preceding the appearance of amyloid and tau pathology. Additionally, we observed alterations in axonal and myelin sheath ultrastructure in the brains of 3xTg-AD mice using electron microscopy. Currently, we are assessing the fate of oligodendrocyte cell lineage in response to AD-related insults. **Conclusions:** Overall, these findings indicate that 3xTg-AD mice represent a viable model in which to examine mechanisms underlying AD-related myelination and neural transmission defects that occur early during pre-symptomatic stages of the disease process. Our findings also suggest that oligodendrocytes represent vulnerable cellular targets that may dictate the regional and temporal nature of disease progression. Future studies will provide valuable insight into the involvement of white matter disruption in the temporal and spatial progression early AD pathogenic events and may illuminate new therapeutic strategies designed to avert early AD-related impairments resulting from oligodendrocyte and myelin aberrations.

P3-405 OVEREXPRESSION OF HUMAN APP INCREASES NEURONAL CALCIUM INFLUX THROUGH L-TYPE CALCIUM CHANNELS INDEPENDENTLY OF Aβ OR γ-CLEAVAGE

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Background: Calcium homeostasis is crucial for neuronal survival. A growing body of evidence shows that in Alzheimer disease, calcium homeostasis is disrupted, leading to abnormal synaptic function and memory deficits. Voltage-dependant calcium channels (VDCC) are influenced by Aβ and presenilins, but little is known about the contribution of the Amyloid Precursor Protein (APP) to calcium currents in neurons. **Methods:** Overexpression of human neuronal APP (hAPP) in primary cultures of rat cortical neurons was mediated by recombinant adenoviruses. Voltage-dependent calcium channels activity was recorded by the patch-clamp technique in the whole-cell configuration and cytosolic calcium concentration was measured using FURA-2. The density of L-type VDCC at the cell surface was measured in binding experiments using a radiolabelled ligand, [^{3}H]PN200-110. **Results:** BayK6844, a L-type calcium channel agonist, induced a larger increase in cytosolic calcium concentration in neurons expressing hAPP than in control (β-gal expressing) neurons. The increased response to BayK6844 was maintained after inhibition of Aβ production and γ-cleavage by DAPT, a functional inhibitor of the γ-secretase activity. The effect of APP could not be attributed to an increased number of L-type calcium channels at the cell surface, as measured by the binding of [^{3}H]PN200-110 to hAPP or β-gal expressing neurons. Depolarization of neurons using 50mM KCl led to a similar increase in cytosolic Ca^{2+} concentration in hAPP or β-gal expressing neurons. Voltage-clamp recordings showed similar densities of calcium currents in hAPP expressing neurons, when compared to non-infected neurons. However, a shift of the voltage dependence of activation to more positive potentials was observed, which is compatible with a higher contribution of L-type calcium channels to the global calcium current. **Conclusions:** Neuronal expression of human APP increases the activity of L-type calcium channels, independently of Aβ production or γ-cleavage. This increase does not induce a higher total calcium current, contrary to previous observations, after treatment of neurons with synthetic Aβ. An important function of APP could be to regulate voltage-dependent calcium channels by favoring the L-type calcium channel contribution to total calcium currents.

P3-406 MATRIX METALLOPROTEINASE–MEDIATED CLEAVAGE OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN TO GENERATE SOLUBLE SPECIES WITH NEURODEGENERATIVE POTENTIAL

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Background: The low-density lipoprotein receptor-related protein (LRP) is a large multi-functional endocytic scavenger receptor capable of internalizing of over 50 ligands. These include molecules important in Alzheimer's disease (AD) such as the β-amyloid peptide and apolipoprotein E. Like other membrane-bound receptors, LRP can be proteolytically cleaved within the ectodomain to generate soluble species (sLRP). The soluble species retain the ability to bind ligands but are unable to internalize them and so may function as competitive antagonists of the membrane-bound receptor in regard to ligand uptake. Therefore, cleavage of membrane-bound LRP and enhanced sLRP generation might affect the turnover of molecules important in AD. The