P4-033 MELATONIN ATTENUATES Aβ AMYLOIDOSIS INDUCE TAU HYPERPHOSPHORYLATION IN TRANSGENIC MICE TG2576 OF DIFFERENT AGES

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Background: Key pathological hallmarks of Alzheimer's disease (AD) are the deposition of amyloid plaques containing Aβ-peptides and the formation of neurofibrillary tangles containing hyperphosphorylated tau. APPsw transgenic mice (Tg2576) overproducing mutant amyloid β protein precursor (βAPP) show substantial brain Aβ amyloidosis and behavioural abnormalities. In Tg2576, Aβ deposits induce phosphorylated tau accumulated at 8-10 months. Phosphorylated tau at Ser199, Thr231/Ser235, Thr205 and Ser404 accumulated in different ages. The major kinase for tau phosphorylation was GSK3β and CDK-5. The pineal and retinal melatonin regulates endogenous circadian rhythms, and has various physiological functions including neuromodulatory and vasoactive actions, antioxidative and neuroprotective properties. Our recent studies have demonstrated that melatonin efficiently attenuates Alzheimer-like tau hyperphosphorylation in Tg2576. **Methods:** Four-month-old (n=4-5)and Eight-month-old (n=4-5) Tg2576 animals (Tgs) were daily injections of melatonin (14mg/kg) for 4 months. Respective controls included non-transgenic littermates (Lts) (n=4-5), and untreated Tgs (n=4-5). After 4 months of treatment, Hyperphosphorylated tau, GSK-3β and CDK5 were determined by Western blotting, immunohistochemistry with specific antibodies. **Results:** The long-term influence of melatonin on behavior, biochemical and neuropathologic changes in Tg2576. In Eight-month-old mice, Hyperphosphorylated tau epitopes were substantially decreased as assessed with the pT205 and pS404 antibodies in mel-treated Tg mice. The untreated Tg mice show increased levels of tau hyperphosphorylation and increased activated CDK5. In twelve-month-old mice, Hyperphosphorylated tau were substantially decreased as assessed with the pT231 antibodies in mel-treated Tg mice. The untreated Tg mice show, together with increased amyloidogenesis, increased levels of tau hyperphosphorylation and increased activated GSK-3β. Four months of Melatonin treatment reduced the burden of amyloid plaques and the levels of hyperphosphorylated tau. Melatonin reduced the activated CDK5 and GSK-3β respectively in 8 months and 12 months. **Conclusions:** Melatonin can exert multiple protective effects on both amyloidogenesis and tau hyperphosphorylation via regulate the activated CDK5 and GSK-3β in transgenic mice Tg2576 of different ages.

P4-034 DIETARY INDUCTION OF TYPE 2 DIABETES INCREASES ALZHEIMER-LIKE PATHOLOGY IN MALE 3XTG-AD MICE

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Background: Metabolic syndrome and type 2 diabetes (T2D) have been identified as risk factors for the development of Alzheimer's disease (AD). While it remains unclear how these conditions are related, both T2D and AD are amyloidogenic disorders with pathological process that are characterized in part by involvement of common signaling pathways and molecules. For example, there are similarities in the regulation of amyloid pathology in T2D and AD, including the amyloid catabolizing enzymes insulin degrading enzyme and neprilysin. Further, both diseases involve dysregulation of glycogen synthase kinase-3β pathways. **Objective:** Our goal was to begin examining the effect of T2D-like metabolic changes on the development of AD pathology. Specifically, we compared two dietary strategies known to induce T2D-like metabolic changes, elevated levels of sucrose water and high fat, on behavioral changes and AD pathology in 3xTg-AD mice. **Methods:** Male 3xTg-AD mice were given either normal diet, 10% sucrose-supplemented drinking water, or a 60% high fat diet beginning at age 3 or 13 months, then maintained on these diets for 4 months. At age 7 and 17 months, mice were evaluated for metabolic changes, behavioral deficits, and accumulation of β-amyloid (Aβ) and hyperphosphorylated tau. **Results:** We observed that both high sugar and high fat diets induced metabolic changes associated with metabolic syndrome. Further, both diets accelerated onset of AD-like neuropathology and behavioral impairments in comparison to 3xTg-AD mice with normal diet. **Conclusions:** These data demonstrate the validity and utility of dietary induction of T2D-related metabolic changes to study potential interactions between T2D and AD. Importantly, our findings also support the hypothesized interaction between T2D metabolic changes and promotion of AD neuropathology.

P4-035 TEMPORAL PROFILE FOR AMYLOID PRECURSOR PROTEIN, AMYLOID-β AND TAU EXPRESSION IN PRIMARY CULTURES FROM 3XTG-AD MICE

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Background: Advances in transgenic technology, as well as in the genetics of the early-onset familial form and the genetic risk factors involved in sporadic cases of Alzheimer disease (AD) have been critical for establishing animal models that reproduce amyloid-beta plaques and neurofibrillary tangles, the main pathological hallmarks of AD, useful for assessing new preventive or therapeutic approaches prior to the initiation of human clinical trials. In the year 2003, the development of triple-transgenic 3xTg-AD mice harboring PS1/M146 V, APPSwe and tauP301L transgenes (Oddo et al.) provided an unique model that closely mimics AD features in the human patient brain. **Methods:** ''In vitro'' primary cortical neuronal cultures obtained from 3xTg-AD mice and NonTg-AD mice. Immunocytochemistry combined with confocal microscopy and western blot analysis to assess the levels of APP, beta-amyloid and tau. **Results:** We have characterized the feasibility and temporal profile of the main pathologic hallmarks of AD (overexpression of APP, amyloid-beta peptide and tau) in an ''in vitro'' primary neuronal cultures model obtained from 3xTg-AD mice and compared the results with the expression of these proteins in the same cultures from non 3xTg-AD mice. As evidenced by inmunocytochemistry combined with confocal microscopy, primary cultures of cortical neurons obtained from 3xTg-AD mice showed overexpression of APP, extracellular amyloid-beta peptide, total Tau and three different phosphorylated isoforms of Tau in a developmental manner. Western blot analysis of the levels of Tau and amyloid-beta peptide in cultures from 3xTg-AD and non 3xTg-AD mice corroborated the results obtained in inmunocytochemistry experiments. In addition primary neuronal cultures from 3xTg-AD mice showed alterations in cytsolic calcium homeostasis, decreased cholinergic and glutamatergic responses. **Conclusions:** This work show that primary neuronal cultures from 3xTg-AD mice provide the first ''in vitro'' model with simultaneous overexpression of APP, Tau and intra and extracellular amyloid-beta and could constitute a valuable model for evaluating potential AD therapeutics as the impact on both Tau and amyloid-beta peptide can be easily assessed in vitro. Work supported by Ministerio Educación y Ciencia SAF2006-13642 and Xunta de Galicia, PGIDT/PGIDIT 07CSA012261PR.

P4-036 AGE-RELATED IMPAIRMENTS IN TAU TRANSGENIC MICE USING TWO DIFFERENT WATER MAZE TRAINING PROTOCOLS

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Background: Intracellular inclusions of filamentous tau are neuropathological hallmarks of neurodegenerative diseases collectively known as tauopathies, which are associated with the progressive loss of cognitive, behavioural and

motor functions. **Methods:** Line 1 mice over-expressing a gene coding for human truncated tau (amino acids 295-390 combined with an N-terminal signal sequence) were bred on an NMRI background. Female, homozygous transgenic mice aged ~4 and 11 months were assessed in two different open field water maze paradigms and compared with age-matched wild-type controls. In the spatial reference memory task (A), the hidden platform is maintained in its location throughout learning. By contrast, in the spatial problem-solving task (B), an initial visual pre-training is followed by training to a hidden platform until a criterion is met, then the platform changes location and animals are trained until the next criterion is met, and then repeated. **Results:** The spatial reference memory task (A) did not reveal any age-related spatial learning deficit since neither elderly controls nor transgenic mice were able to acquire the task. Given the progressive accumulation of tau in the transgenic mice during ageing, however, one would expect them to perform worse than wild-type animals. In the alternative, problem solving task (B), a cognitive impairment in NMRI-derived transgenic mice was observed. Although aged mice were impaired visually and motorically in the visible platform test relative to young mice, there was no genotype difference. When trained to criterion, tau transgenic mice required longer to learn the location of the platform and, over a set acquisition period, achieved fewer platform locations. **Conclusions:** These findings indicate that there is a learning deficit in these mice that is related to transgene expression but which is not due to visual or motor impairment. The line 1 mouse provides a model for the study of cognitive dysfunction in tauopathies and an important tool for investigating the mechanism underlying neurodegeneration and cognitive impairment in age-related neurodegenerative disease.

P4-037 NOVEL AGE-DEPENDENT LEARNING DEFICITS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE: IMPLICATIONS FOR TRANSLATIONAL RESEARCH

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Background: The ability to accurately apply previously learned information to novel problems and situations has been referred to as reconfiguration of stimuli or 'transfer learning'. Previous studies have shown that hippocampal damage impairs such abilities in rodents and nonhuman primates. Moreover, in humans, mild hippocampal atrophy in the elderly correlates with such deficits and may even predict progression into dementia. However, theories and models regarding this type of hippocampal function have not been applied to animal models of Alzheimer's disease that are associated with hippocampal dysfunction despite the fact that such learning offers direct translational advantages compared to conventional human and rodent cognitive assessments(e.g. delayed paragraph recall vs. Morris water maze). **Methods:** In this study, a task was developed to assess ''transfer learning'' in mice and specifically to determine if learning was impaired in a mouse model of Alzheimer's disease [AD; APPswe, PSEN1-dE9)85Dbo/o (APP+PS1 mice)]. Mice learned a series of concurrent discriminations in one dimension (odors or digging media) in the presence of a feature-irrelevant stimulus in the other dimension not predictive of reward. This irrelevant stimulus was then changed and discrimination performance evaluated. We hypothesized that mice with hippocampal dysfunction would be able to learn initial discrimination problems but would be impaired in their ability to apply this learned information when the irrelevant stimulus was changed (i.e., the context was reconfigured). **Results:** Three month old APP+PS1 mice were not impaired in initial discrimination learning or on the ability to transfer this learned information to the altered context. In contrast, at 12 months of age, APP+PS1 mice learned the initial concurrent discriminations on par with NTgs but were impaired when required to ''transfer'' this learning into a new configuration/context. There were no differences in Morris water maze performance between the APP+PS1 and NTgs at 12 months of age. **Conclusions:** These data are the first to demonstrate deficits associated with reconfiguration of stimuli or transfer learning thought to be dependent on the hippocampal formation is impaired in a mouse model of AD. Moreover, these data suggest that this deficit may precede or is more sensitive in detecting deficits than water maze in this model.

P4-038 TRANSGENIC MICE WITH THE ARCTIC APP MUTATION SHOW AN AGE-DEPENDENT INCREASE IN Aβ LEVELS

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Background: The pathological mechanisms by which the Arctic *APP* mutation (E693G) within the amyloid-beta (Aβ) peptide cause dementia has not been clarified, but *in vitro* studies have demonstrated that the Arctic mutation alters the properties of the Aβ peptide, resulting in formation of protofibrils more rapidly and at higher levels compared to wild-type Aβ. We have generated a transgenic mouse model expressing human *APP*695 with the Arctic mutation (TgAPParc), which is the only reported *in vivo* model characterizing the effect of the Arctic *APP* mutation without the influence of other *APP* mutations. Heterozygous TgAPParc mice show amyloid deposition in thalamus and subiculum, starting at 7 months of age, which is associated with impaired spatial learning and memory at 15 months in female transgenic mice. Here we report a biochemical characterization of Aβ levels in transgenic mice expressing hAPParc at about a 3-fold level compared to mouse endogenous APP. **Methods:** Aβ40 and Aβ42 levels were measured by ELISA in diethylamine (DEA)-soluble and formic acid (FA)-soluble brain extracts from wildtype and transgenic littermates at different ages (6, 12, 18 and 24 months of age). **Results:** The levels of human Aβ40 and Aβ42 were below the limit of detection in the DEA-soluble brain extracts. However, the TgAPParc mice show an age-dependent increase in both Aβ40 and Aβ42 in FA-soluble brain extracts, starting around 12 months of age. **Conclusions:** In summary, our findings show that a relatively low level of hAPParc overexpression is sufficient to cause an age-dependent increase in both Aβ40 and Aβ42 in FA-soluble brain extracts, suggesting that the majority of Aβ in the brains of the TgAPParc mice is highly aggregated. This is in line with the previous *in vitro* reports of increased protofibril formation caused by the Arctic *APP* mutation.

P4-039 OVEREXPRESSION OF MUTANT TAU IN MICE LEADS TO TAU AGGREGATION AND DETERIORATION OF MOTOR SKILLS

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Background: Tau is a predominantly axonal protein in neurons, where it plays a role in microtubule assembly and stabilisation. Mutations in the tau gene are associated with hereditary fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) and enhance the capacity for the protein to form aggregates *in vitro*. Aggregated tau proteins are also found in a variety of other neurodegenerative diseases including Alzheimer's disease (AD), in which they form the paired helical filaments that constitute the neurofibrillary lesions. **Methods:** To model tau aggregation *in vivo*, a transgenic mouse model (line 66) expressing full-length tau carrying a double mutation (P301S/G335D) under the control of the murine Thy1-promoter was generated on an NMRI background. Brain sections were stained using Bielschowsky silver stain and Thioflavin S and immunohistochemically with mAb 7/51. Homozygous and heterozygous mice together with wild-type NMRI were assessed in a number of behavioural tasks: the SHIRPA screen, open field, rotarod, balance beam, catwalk and grip strength meter. **Results:** The expression of the mutant tau was associated with a prominent and progressive motor impairment in line 66 mice. At 4 months, homozygous mice show decreased speed and locomotor