Author

Throughout a freshman course on neurobiology of disease, Elaine Le was intrigued at how neuroscience delved into the mind and its interaction with the universe. She became fascinated with the many central nervous system processes that affect millions of people with neurological disorders and decided to get involved in neurobiological research. She spent the next several years working in Dr. LaFerla's lab, looking to develop new models to better understand Alzheimer's disease and to improve therapeutic strategies to combat the disease. After graduation, Elaine will seek to experience more fields of science and look for work in a clinical setting to observe treatment of patients outside of the laboratory.

Key Terms

- 3xTg-AD
- Aβ
- Alzheimer's Disease
- Amyloid Cascade Hypothesis
- mtauKO
- NFTs
- PHF
- tau

Endogenous tau Contributes to Alzheimer-Like tau Pathology in 3xTg-AD Mice

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Abstract

Recent critical studies using tauopathy models suggest that the concomitant expression of endogenous murine tau may interfere with the disease progression by delaying the pathological accumulation of human tau in the brain. To understand the role of endogenous murine tau in the pathological events that occur in Alzheimer's disease (AD) models, we developed a novel transgenic mouse model by crossing 3xTg-AD with mtauKO mice, referred to as 3xTg-AD/mtauKO mice. This new model allows us to determine the pathological interaction of murine tau in comparison with its parental line. Here, we show that 3xTg-AD mice presented higher tau loads in both soluble and insoluble fractions and increased tau hyperphosphorylation in the soluble fraction when compared with the parental 3xTg-AD/mtauKO. These results indicate that mouse tau is hyperphosphorylated and significantly co-aggregated with human tau in 3xTg-AD mice. Additionally, both transgenic models showed similar tau-phosphorylation-related kinase activity as well as comparable Aβ pathology. Notably, both models exhibited equivalent cognitive dysfunction when tested on a spatial memory task, indicating that paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) increases observed in 3xTg-AD versus 3xTg-AD/mtauKO mice do not contribute to cognitive impairment.

Faculty Mentor

This manuscript provides relevant insights to better understand the contribution of endogenous tau in AD pathology, and emphasizes the necessity of developing new AD models that better reproduce human AD pathological events.

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Introduction

Alzheimer's disease (AD) is the most common form of dementia that leads to progressive deterioration of memory and cognition among elderly populations. Aging is one of the main factors that contribute to AD pathology; after the age of 65 the risk of developing AD significantly doubles every five years in the human population. Thus, by the age of 85, one in three individuals is subject to developing AD. More than 35 million people throughout the world are afflicted, including 5.4 million in the USA. With no current cure for this neurodegenerative disorder, it is estimated that the prevalence of AD will quadruple by the year 2050 (Brookmeyer et al., 2007).

Pathologically, AD is characterized by extracellular accumulation of amyloid-β (Aβ) plaques and intracellular aggregation of neurofibrillary tangles (NFTs). The prevailingly accepted amyloid cascade hypothesis suggests that altered proteolytic cleavage of amyloid precursor protein (APP) and clearance of the generated Aβ species results in an imbalance of Aβ production and progressive accumulations in the brain (Hardy and Selkoe., 2002). These Aβ accumulations can modulate and trigger the formation of NFTs, which is constituted by aggregates of a protein called tau. In this regard, numerous studies carried out by our group and others support the hypothesis of a modulatory interaction between Aβ and tau, which includes the evidence that Aβ triggers the onset and progression of tau pathology (Gotz et al., 2001; Lewis et al., 2001; Oddo et al., 2008; Tseng et al., 2008). Moreover, recent studies indicate that tau is a critical downstream target through Aβ-amyloid peptide induced neurodegenerative processing (Rapoport et al., 2002; Roberson et al., 2007; Vossel et al., 2010; Shipton et al., 2011). These findings from animal studies in part support clinical observations that tau pathology better correlates in severity of cognitive impairments than Aβ pathology in AD, and that high amyloid loads, but not tau, are sometimes detected in aged, non-demented individuals. Therefore, abnormal tau accumulations may closely link to neurodegenerative diseases associated with dementia.

The microtubule-associated protein tau (MAPT) is a neuron-specific cytoskeletal protein that is directly involved in maintaining the integrity, stability and dynamics of microtubules (Terwel et al., 2002; Medeiros et al., 2011). Microtubules are dynamic structures and are important components of the cytoskeleton. They also have relevant functions in determining cell shape and polarity that are essential key factors in the formation of axon and dendrite structures in neurons. Moreover, recent evidence suggests that tau has an important role in signal transduction mechanisms, actin cytoskeleton interactions, and neurite outgrowth and stabilization during brain development (Denk and Wade-Martins, 2009). Tau usually exists as an unfolded and highly soluble protein in the brain. However, in several neurodegenerative disorders, tau becomes characteristically insoluble and aggregated in selected brain regions. These pathological changes are observed in AD and other human tauopathies such as Pick's disease (PiD), progressive spranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease and fronto-temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (Hernandez and Avila, 2007).

Despite current intensive research, the molecular mechanisms on the development and progression of tau pathology and its interaction with Aβ remain unknown. Interestingly, an elimination of the endogenous murine tau expression prevents lethality and cognitive deficits observed in AD transgenic mice (Roberson et al., 2007). In addition, tau has been shown to be involved in Aβ-induced axonal defects, long-term potentiation deficits, and synaptotoxicity (Vossel et al., 2010; Shipton et al., 2011). On the other hand, several studies have shown that the presence of the endogenous murine tau in transgenic mice overexpressing human wildtype or mutant tau exhibits delayed pathological tau accumulations mainly composed of human tau transgenes. When the endogenous murine tau is removed from transgenic tau mice, the onset and the progression of AD-like NFTs and paired helical filament (PHF) aggregates are accelerated (Andorfer et al., 2003; Ando et al., 2011). These studies suggest that endogenous murine tau interferes with human tau aggregation and toxicity by unknown mechanisms.

To better understand the pathological mechanisms of AD in transgenic mice, we generated an AD mouse model in the absence of endogenous murine tau. To achieve this goal, we used a triple transgenic mouse model expressing both AD pathologies, termed 3xTg-AD. We then genetically crossed these mice with mouse tau knockout (mtauKO) mice—mice that do not express endogenous mouse tau—obtained from Dr. Vitek's laboratory (Dawson et al., 2001). This novel transgenic line, referred to as 3xTg-AD/mtauKO, was hypothesized to exhibit more accurate molecular interactions between Aβ and tau from human origin and pathological processing of AD without having any interference from murine tau. Interestingly, the 3xTg-AD mice showed increased levels of tau hyperphosphorylation, PHF assembly, and NFT formation versus 3xTg-AD/mtauKO mice. These increases in PHF assembly and NFT formations observed in the parental line (3xTg-AD)

are not associated with impairment or cognitive dysfunction in comparison with 3xTg-AD/mtauKO mice. Our study suggests that endogenous murine tau contributes to the pathological events that occur in 3xTg-AD mice developing NFTs. However, these increases in NFTs are not correlated to further cognitive impairment.

Material and Methods

Transgenic Mice

The characterization of 3xTg-AD mice has been described previously (Oddo et al., 2003). Briefly, two independent transgenes encoding human APP_{Swe} and the human tau_{P301L} (both under control of the mouse Thy1.2 regulatory element) were co-microinjected into single-cell embryos harvested from homozygous mutant $PS1_{M146V}$ knockin (PS1-KI) mice (Oddo et al., 2003). We crossed these 3xTg-AD mice with tau knockout mice generated in the laboratory of Dr. Vitek (Dawson et al., 2001). Through careful breeding, we derived a colony of 3xTg-AD mice that lack both copies of the endogenous mouse wild type tau gene (i.e., $hPS1KI_{M146V}$/$hPS1KI_{M146V}$; $hAPP_{SWE}$/ $hAPP_{SWE}$; $hTau_{p301l}$/ $hTau_{P301L}$; $hTau_{P301L}$; mTau-/-; where h=human and m=mouse gene). We refer to these mice as the 3xTg-AD/mtaukO. In this study 12- and 18-month-old homozygous nontransgenic (Ntg), mouse tau knockout (mtauKO), triple transgenic AD (3xTg-AD), and crossed triple transgenic tau knockout (3xTg-AD/mTauKO) mice, with 16–20 individuals per group (males and females) were used. All animal procedures were performed in accordance with National Institutes of Health and University of California guidelines and the Institutional Animal Care and Use Committee at the University of California, Irvine.

Behavioral Test

Hidden Morris water maze tests were conducted as described previously (Billings et al., 2005). Mice were trained to swim to a 14 cm diameter circular Plexiglas platform submerged 1.5 cm beneath the surface of the water that was invisible to the swimming mice. The platform was located in a fixed position, equidistant from the center and the wall of the tank. Mice were subjected to four training trials per day. During each trial, mice were placed into the tank at one of four designated start points in a pseudorandom order. Mice were trained for as many days as needed to reach the training criterion of 25 sec (escape latency). If the mice failed to find the platform within 60 sec, they were manually guided to the platform and allowed to remain there for 5 sec. The probe trial was assessed 24 hr after the last training session and consisted of a 60 sec free swim in the pool without the platform. Performance was monitored with the EthoVision XT video tracking system.

Tissue Preparation

After deep anesthesia with sodium pentobarbital (60mg/kg), 12- and 18-month-old Ntg, mtauKO, 3xTg-AD and 3xTg-AD mice were perfused transcardially with 0.1M phosphate-buffered saline (PBS), pH 7.4. Half of the brain was fixed for 48 hr in 4% paraformaldehyde in 0.1M phosphate buffer saline, pH 7.4 and cryoprotected in 30% sucrose for immunohistochemical analysis, whereas the other half was frozen in dry ice for biochemical analysis. Thick (40 μm) free-floating sections were obtained using a freezing microtome (Leica SM 2010 R) and serially collected (each series contained sections that represented 1/7 of the total brain) in cold PBS and 0.02% sodium azide.

Protein extracts were prepared by homogenizing whole brain hemisphere samples in T-per (Pierce) extraction buffer (150mg/ml), complemented with proteases (Complete Mini Protease Inhibitor Tablets, Roche) and phosphatases inhibitors (5mmol/L sodium, Sigma), followed by centrifugation at 100,000 x g for 1 hr. Protein concentration in the supernatant was determined using the Bradford assay.

Acetone/TCA precipitation protocol was used for insoluble protein extraction and analysis (Fic et al., 2010).

Immunohistochemistry

Coronal free-floating sections (40 μm thick) were pretreated with 3% H_2O_2/3% methanol in Tris-buffered saline (TBS) for 30 min to block endogenous peroxide activity. After the TBS wash, sections were incubated once in TBS with 0.1% Triton X-100 (TBST) for 15 min and once in TBST with 2% bovine serum albumin (BSA, Sigma-Aldrich) for 30 min. Sections were incubated with one of the following primary antibodies: anti-6E10 (1:1000, Signet) in TBS+5% normal goat or horse serum overnight at 4 °C. Sections were then incubated with the appropriate secondary antibody (biotinylated anti-rabbit or anti-mouse; 1:500 in TBS+2%BSA+5%normal serum) for 1h at 20 °C, followed by Vector ABC Kit and DAB reagents (Vector Laboratories) to visualize staining. Sections were mounted on gelatin-coated slides, dehydrated in graded ethanol, cleared with xylene and cover-slipped with DPX (BDH) mounting medium. The immune reactions were controlled by omitting the primary antibody.

Immunofluorescence

Coronal free-floating sections were pretreated with 3% H_2O_2/3% methanol in Tris-buffered saline (TBS) for 30

min to block endogenous peroxide activity. After the TBS wash, sections were incubated once in TBS with 0.1% Triton X-100 (TBST) for 15 min and once in TBST with 2% bovine serum albumin (BSA, Sigma-Aldrich) for 30 min. Sections were incubated with one of the following primary antibodies: anti-PHF1 (Dr. Peter Davies, Albert Einstein College of Medicine, Manhasset, NY, USA) in TBS+5% normal goat or horse serum overnight at 4 °C. Sections were then rinsed and incubated for 1 hr with secondary Alexa Fluor-conjugated antibody (Invitrogen, Carlsbard, CA, USA) at room temperature. Finally, sections were mounted onto gelatin coated slides in Fluoromount-G (Southern Biotech, Birmingham, AL, USA) and examined under a Leica DM2500 confocal laser microscope.

Gallyas Silver Staining

Conventional Gallyas silver staining was performed to identify hyperphosphorylated tau aggregates (Gallyas F., 1971; Braak et al., 1988).

Immunoblotting

Equal amounts of protein (20–50 μg, depending on protein of interest) were separated on 4–12% Bis-Tris gel (Invitrogen, Carlsbad, CA), transferred to 0.45 μmol/L polyvinylidene difluoride membranes. Membranes were blocked for 1 hr in 5% (w/v) suspension of nonfat milk in 0.2% Tween 20 Tris-buffered saline (pH 7.5). After blocking, the membranes were incubated overnight, at 4 °C, with one of the following primary antibodies: anti-6E10 (1:1000, Signet), CTF20 (1:5000; Calbiochem) for C99 and C83, HT7 (1:5000; Pierce Biotechnology), AT8 (1:1000; Pierce Biotechnology), AT100 (1:1000; Pierce Biotechnology), AT180 (1:1000; Pierce Biotechnology), AT270 (1:1000; Pierce Biotechnology), PHF1 (Dr. Peter Davies, Albert Einstein College of Medicine, Manhasset, NY, USA), Poly-Tau (1:3000; dako), anti-pGSK3β(ser9) (1:1000; Cell Signaling), anti-Cdk5 (1:1000; Calbiochem), anti-C'-term p35 (1:200; Santa Cruz Biotechnology) for p25 and p35, GAPDH (1:5000, Santa Cruz Biotechnology). The membranes were washed in tween-TBS for 20 min and incubated at 20 °C with the specific secondary antibody at a dilution of 1:10000 (Pierce Biotechnology) for 60 min. The blots were developed using Super Signal (Pierce Biotechnology).

Enzyme-linked Immunosorbent Assay for $A\beta_{40}$ and $A\beta_{42}$

$A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were measured using a sensitive sandwich ELISA system as previously described (Green et al., 2006).

Quantitative and Statistical Analyses

All immunoblot data were quantitatively analyzed using Image J 1.4 software. The data were subsequently analyzed by Student's *t*-test comparison using Graphpad Prism software (Graphpad Prism Inc., San Diego, CA, USA). The significance was set at 95% confidence. All values are presented as mean ± SEM.

Results

Endogenous Mouse Tau Deletion in 3xTg-AD Mice

To study pathological involvements of the endogenous murine tau in a transgenic mouse model of AD with plaques and tangles, we crossed the 3xTg-AD mice with mtauKO mice. The resulting transgenic mice (3xTg-AD/mtauKO) harbored APPswe (+/+), PS1M146V (+/+), tauP301L (+/+) and no expression of murine endogenous tau (-/-), enabling us to examine the pathological interactions and involvements of the murine endogenous tau by directly comparing with its parental line, 3xTg-AD mice. Western

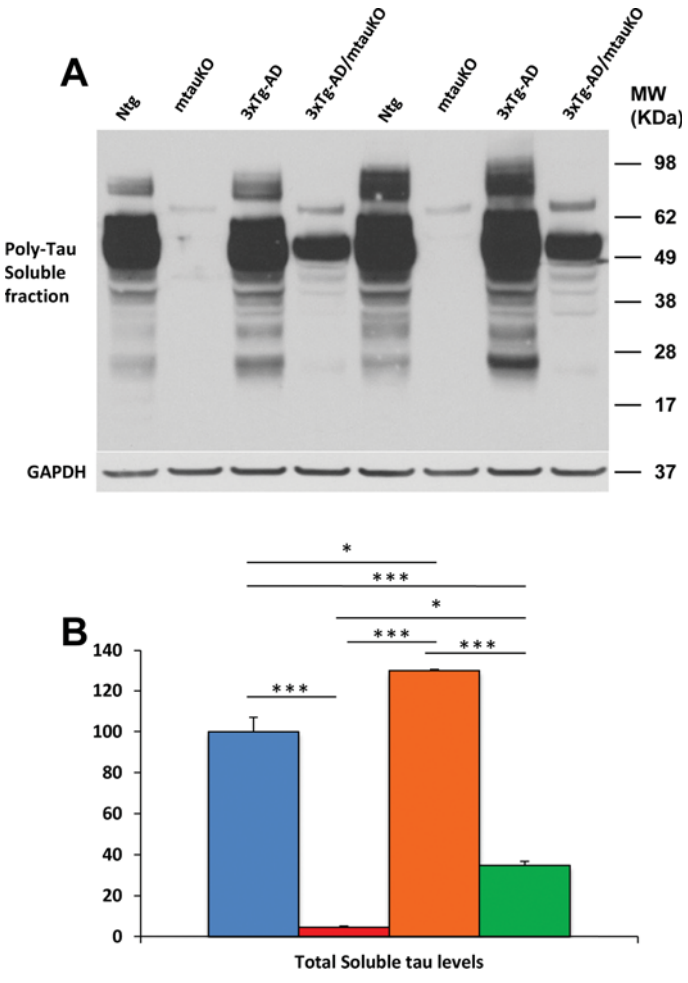

Figure 1

Tau protein levels. Immunoblot showing tau protein levels from whole-brain homogenates of Ntg, mtauKO, 3xTg-AD and 3xTg-AD/mtauKO. The values represent the mean ± S.E.M. (N = 4). *p < 0.05, **p < 0.01 and *** p < 0.001.

blot analyses showed complete absence of steady-state levels of endogenous tau in mtauKO (Figure 1). Furthermore, we found reductions of tau steady-state levels in 3xTg-AD/mtauKO mice compared to 3xTg-AD mice (Figure 1). These results are consistent with deletion of endogenous murine tau in 3xTg-AD/mtauKO animals.

Endogenous Mouse Tau Deletion Does Not Alter Hippocampal Cognitive Dysfunction in 3xTg-AD Mice

To address the impact on cognition of endogenous mouse tau, Ntg, mtauKO, 3xTg-AD and 3xTgAD/mtauKO mice were tested on a spatial hippocampal-dependent behavior task, the Morris water maze (MWM). Mice were trained to criterion (escape latency <25 seconds) in the spatial reference version of MWM to find the location of a hidden platform. No significant differences were observed on acquisition memory among the different genotypes (Ntg, mtauKO, 3xTg-AD and 3xTg-AD/mtauKO) at 12 months of age (Figure 2A). However, significant differences were found at 18 months in Ntg and mtauKO mice compared to 3xTg-AD and 3xTg-AD/mtauKO mice. Ntg mice reached criterion in four days, mtauKO mice required seven days,

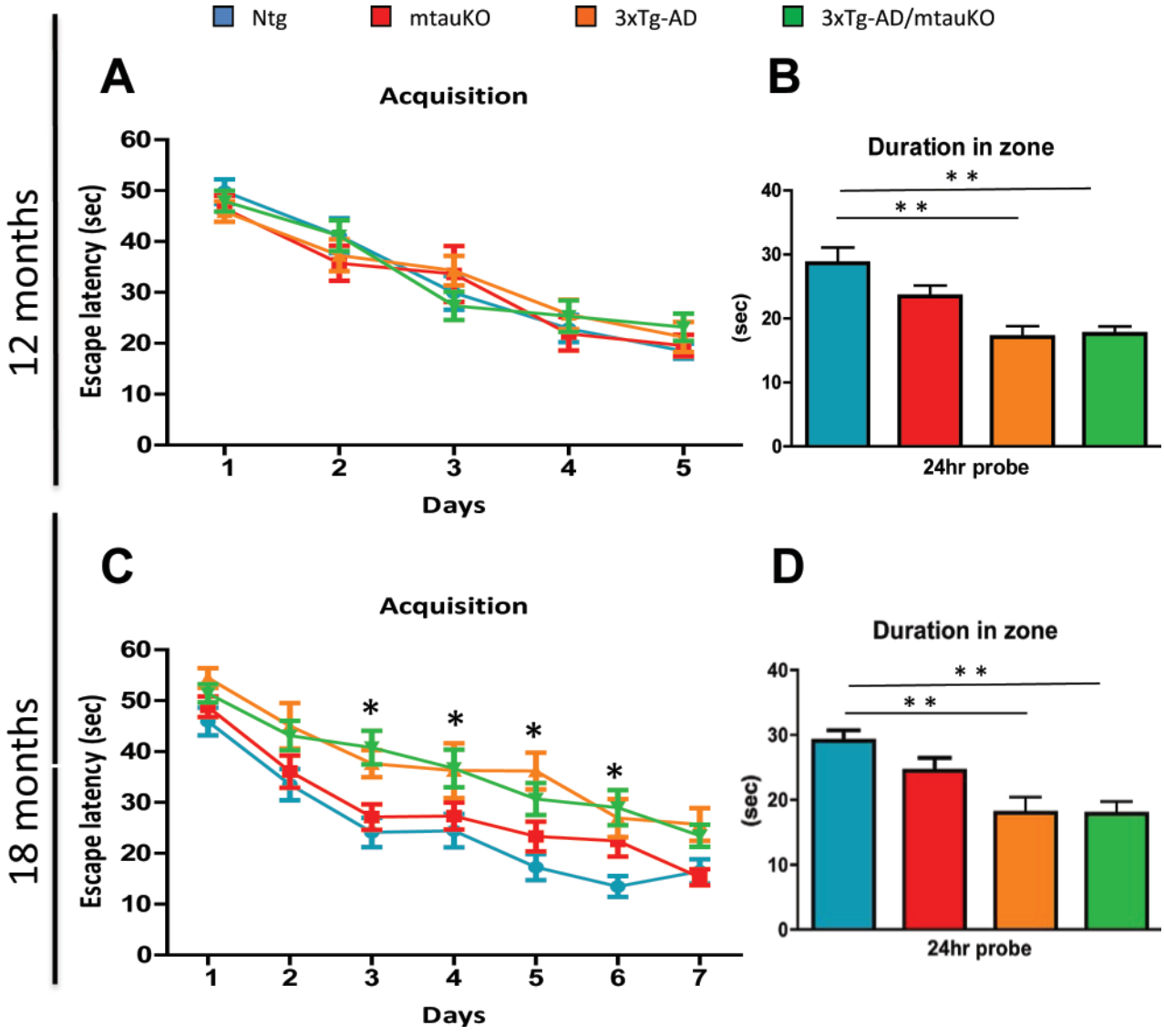

Figure 2

Depletion of mouse tau in the 3xTg-AD mice did not alter hippocampal cognitive impairment. Mice were trained on the spatial reference version of the Morris water maze at 12 (A and B) and 18 (C and D) months of age. (A) No significant differences were observed on the acquisition curves at 12 months of age (five days of training). (C) Significant differences were observed, however, in the 18-month-old mice (seven days of training). Ntg and mtauKO were different when compared with 3xTg-AD and 3xTg-AD/mTauKO. Significant differences were observed between Ntg and 3xTg-AD and between Ntg and 3xTg-AD/mTauKO at 12 (B) and 18 (D) months of age in the time spent in the target zone. The values represent the mean ± S.E.M. (N = 16–20). *p<0.05, **p<0.01.

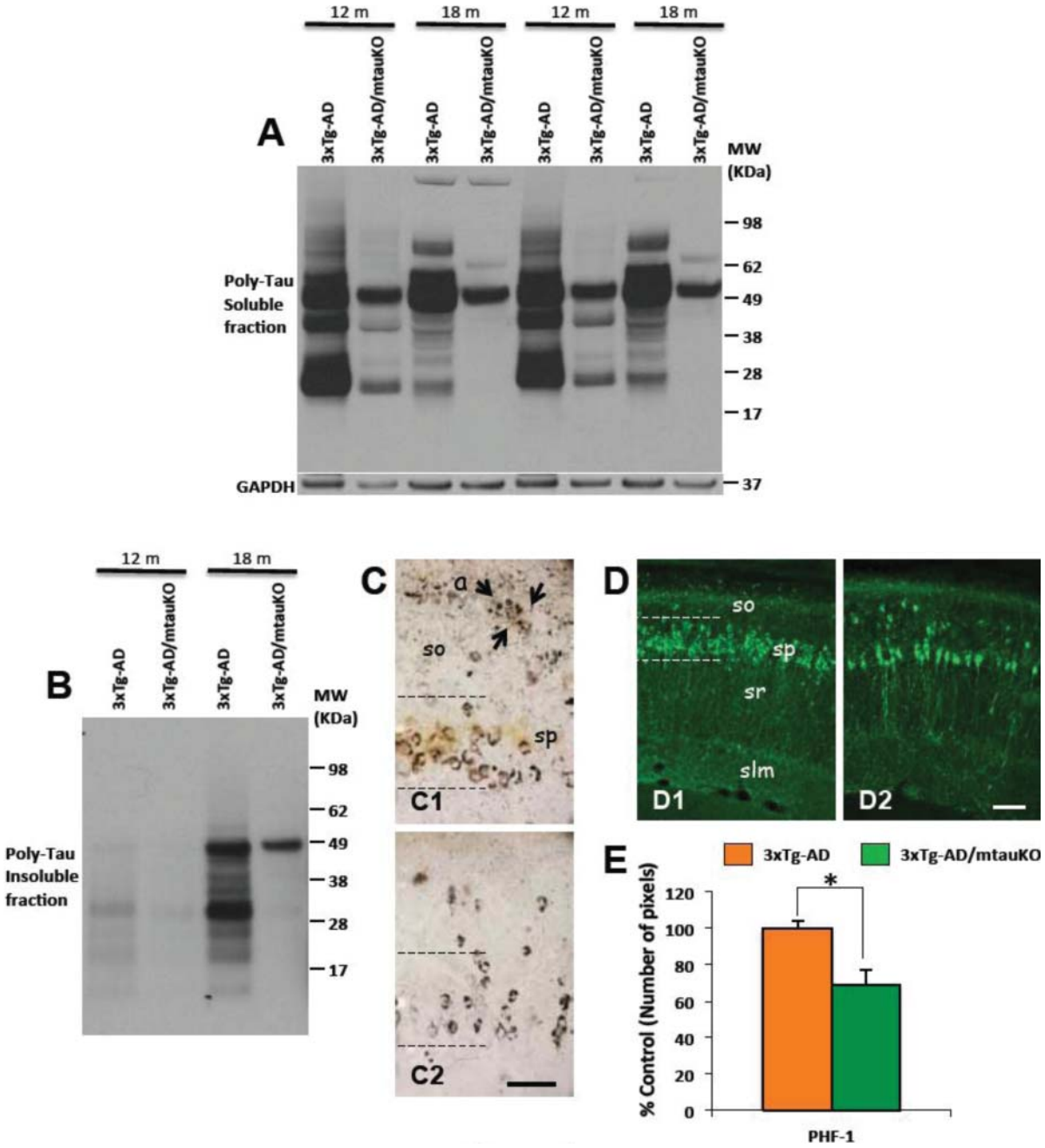

Figure 3

Higher soluble and insoluble tau levels in 3xTg-AD compared to 3xTg- AD/mtauKO mice. Immunoblot analysis of tau expression from soluble (A) and insoluble (B) whole-brain homogenates in 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months of age (n=8), shown as alternating lanes. Age-dependent decrease in soluble tau levels was detected by immunoblot analysis in 3xTg-AD and 3xTg-AD/mtauKO mice. In correlation with soluble tau reduction levels, significant age-dependent increases in the insoluble fraction were observed in both 3xTg-AD and 3xTg-AD/mtauKO mice. (C) Representative hippocampal sections (CA1 subfield) in 3xTg-AD (C1) and 3xTg-AD/mtauKO (C2) of 18-month-old mice, showing intraneuronal NFTs accumulations, as detected by Gallyas silver staining. Significant increases in the number of NFTs detected were observed in 3xTg-AD (C1) compared to 3xTg- AD/mtauKO (C2) mice. The presence of dystrophic neurites (arrows) was observed in the stratum oriens/alveolus of 3xTg-AD mice (C1). (D) Representative hippocampal sections (CA1 subfield) in 3xTg-AD (D1) and 3xTg-AD/mtauKO (D2) at 18 month-old mice, showing mainly somatic PHF accumulation in the 3xTg-AD and somatodendritic PHF accumulation in the 3xTg- AD/mtauKO (a: alveolus, so: stratum oriens, sp: stratum pyramidale, slm: stratum lacunosum-moleculare). (E) Quantification of PHF-1 expression in CA1 hippocampal subfield.. Scale bars: 50 μm (C) and 200 μm (D). The values represent the mean ± S.E.M. *p<0.05.

and neither 3xTg-AD nor 3xTg-AD/mtauKO mice reached criterion after seven days of training. However, when compared between 3xTg-AD and 3xTg-AD/mtauKO mice, no differences were observed in the acquisition memory at this age (Figure 2C).

The retention memory was tested 24 hr after the last training trial. At both ages, we found significant decreases in the time spent in the platform quadrant in 3xTg-AD (at 12 months 35.63±5.92% one way ANOVA, Bonferroni post-hoc **p<0.01 and at 18 months 44.02±8.25% one way ANOVA, Bonferroni post-hoc **p<0.01) and 3xTg-AD/mtauKO (at 12 months 30.32±4.98% one way ANOVA, Bonferroni post-hoc **p<0.01 and at 18 months 30.84±7.51% one way ANOVA, Bonferroni post-hoc **p<0.01) compared to Ntg (Figures 2B and D). Notably, no differences were observed between 3xTg-AD and 3xTg-AD/mtauKO mice at both ages (Figures 2B and D). The above observed differences were not due to apparent motor impairments as swim speed or total travel distance was consistent among the groups (data not shown). These data suggest that endogenous tau deletion in 3xTg-AD mice did not significantly alter cognitive deficits.

3xTg-AD Mice Express Higher Levels of Soluble and Insoluble Tau Compared to 3xTgAD/mtauKO Mice

We next analyzed the soluble and insoluble tau levels in the 3xTg-AD and 3xTg-AD/mtauKO mice to correlate cognitive deficits observed in these mice. Steady-state levels of soluble tau showed similar patterns in the 3xTg-AD and 3xTg-AD/mtauKO mice that only express human tau. Furthermore, significant age-dependent reductions of tau levels were detected mainly at 60, 45 and 28 kDa in both 3xTg-AD and 3xTg-AD/mtauKO mice (Figure 3A). On the other hand, we observed significant age-dependent increases of insoluble tau levels in the 3xTg-AD compared to the 3xTg-AD/mtauKO at 18 months (Figure 3B). Therefore, endogenous tau appeared to co-aggregate with human tau to develop NFTs in 3xTg-AD mice at 18 months of age.

Gallyas silver staining further confirmed an increase in the number of intraneuronal NFTs in the pyramidal cell layer of the hippocampus in 3xTg-AD compared to 3xTg-AD/mtauKO mice (Figure 3C). In addition, a significant number of dystrophic neuritis that displayed a swollen/globular appearance positive for Gallyas silver staining was preferentially located in the stratum oriens of the CA1 hippocampal subfield (Figure 3C1) and subiculum (not shown) in the 3xTg-AD compared to 3xTg-AD/mtauKO at 18 months. These data suggest that an age-dependent reduction of soluble tau levels in both 3xTg-AD and 3xTg-AD/mtauKO groups is correlated with an age-dependent increase in the insoluble tau fraction. In addition, a significantly higher number of dystrophic neuritis was detected in the stratum oriens in the CA1 subfield and the subiculum of the hippocampus in the 3xTg-AD compared to the 3xTgAD-mtauKO group, suggesting a relevant role of endogenous tau in the formation of neurofibrillary tangles. Furthermore, significant numbers of hippocampal pyramidal-cells with somatic accumulation of paired helical filaments were observed in the 3xTg-AD group compared to 3xTg-AD/mtauKO group at 18 months of age (31.01 ± 8.01% at 18 months *p<0.05, *t*-test) (Figures 3D-E). These results suggest that endogenous tau contributed in the formation of NFTs and PHFs observed in the 3xTg-AD mice.

3xTg-AD Mice Express High Levels of Tau Hyperphosphorylation at Residues Thr-181 and Ser396/404 Compared to 3xTgAD/mtauKO Mice

We next examined phosphorylation states of tau in soluble homogenate samples. The study revealed a significant decrease in tau phosphorylated at residues Thr181 (55.16 ± 16.98% at 12 months (*p<0.05, *t*-test and 86.27± 3.06% at 18 months, ***p<0.001, *t*-test recognized by the AT270 antibody) and Ser396/404 (86.70 ± 5.86% at 12 months ***p<0.001, *t*-test and 73.15± 8.21% at 18 months, ***p<0.001, *t*-test recognized by the PHF-1 antibody) in the 3xTg-AD/mtauKO compared to 3xTg-AD mice. Levels of phospho-tau species recognized by antibodies AT8 (Ser199/202), AT100 (Ser214) and AT180 (Thr231) were unaltered in the 3xTg-AD/mtauKO compared to 3xTg-AD mice (Figures 4A-D). The total human tau detected by the antibody HT7 was not different between the 3xTg-AD and 3xTg-AD/mtauKO mice, suggesting that the presence of the endogenous murine tau did not alter the expression of human tau transgene (Figures 4A and C). Therefore, the results suggest that high levels of tau phosphorylation at Thr181 and Ser 396/404 in the 3xTg-AD was related to an increased level of insoluble tau isoforms detected by Western blot, and significant numbers of PHFs and NFTs by immunohistochemistry and Gallyas silver staining (Figures 3C and D). Notably, this increase of tau hyperphosphorylation in 3xTg-AD mice is presumed to be mainly from the presence of endogenous tau instead of human tau due to the lower levels of tau hyperphosphorylation observed in 3xTg-AD/mtauKO compared to 3xTg-AD mice.

No Difference in Kinase Activity Mediates Increases of Tau Phosphorylation in the 3xTg-AD

To elucidate the mechanisms through which 3xTg-AD displays higher levels of tau hyperphosphorylation com-

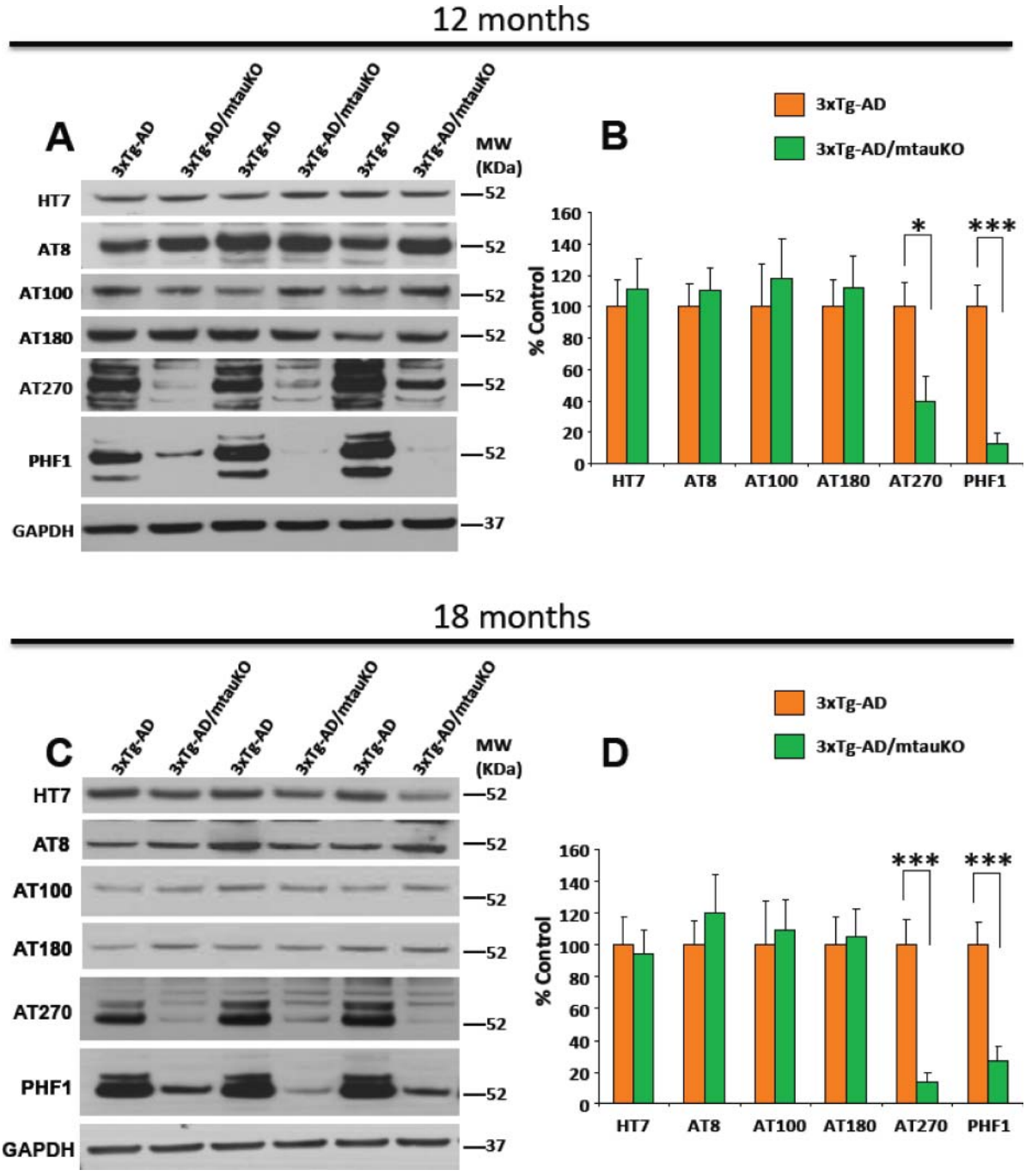

Figure 4

Significant soluble-tau hyperphosphorylation at Thr181 and Ser396/404 was detected in 3xTg-AD compared to 3xTg-AD/mtauKO mice. (A, C) Immunoblot analysis of total tau (HT7) and phospho-tau epitopes—including pSer199/202 (AT8), pThr212/Ser214 (AT100), pThr231 (AT180), pThr181 (AT270) and pSer396/404 (PHF-1)—of protein extracts from whole-brain homogenates of 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months of age (n=8), shown as alternating lanes. (B, D) Quantification of A and C normalized to GAPDH and expressed as a percent of control shows significant decreases in p-tau epitopes at 12 months of age at Thr181 (55.16 ± 16.98%, *p<0.05, *t*-test) and Ser 396/404 (86.70 ± 5.86%, ***p<0.001, *t*-test) and 18 months of age at Thr181 (86.27 ± 3.06%, ***p<0.001, *t*-test) and Ser 396/404 (73.15 ± 8.21%, ***p<0.001, *t*-test) in the 3xTg-AD/mtauKO compared to 3xTg-AD mice. GAPDH levels were used as control for protein loading. The values represent the mean ± S.E.M. *p<0.05 and ***p<0.001.

pared to 3xTg-AD/mtauKO mice, we examined the total and activated forms of the two major kinases involved in tau phosphorylation, cyclin-dependent kinase 5 (cdk5), and Glycogen synthase kinase 3-beta (GSK3β) (Medeiros et al., 2011). The results showed similar steady-state levels of cdk5 and the cytosolic activators of cdk5, p25 and p35 in the 3xTg-AD compared to 3xTg-AD/mtauKO mice at 12 months (Figures 5A-B) and 18 months of age (Figures 5C-D). Similarly, the activation status of GSK3β, detected by the phosphorylation at Ser9 residue as an inactive state (Andorfer et al., 2003), showed no difference in the 3xTg-AD compared to 3xTg-AD/mtauKO mice. The activation of these kinases was not affected by the deletion of the endogenous murine tau, and the decreased tau phosphorylation observed in the 3xTg-AD/mtauKO was mediated by the elimination of the endogenous tau rather than due to an alteration of kinase activities.

Endogenous Tau Deletion in the 3xTg-AD Mice Does Not Alter Aβ Pathology

We further examined whether the deletion of the endogenous murine tau in the 3xTg-AD led to any change in APP processing and/or Aβ deposition. Steady-state levels of full-length APP holoprotein were unaffected by Western blot analysis between 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months of age (Figures 6A and C).

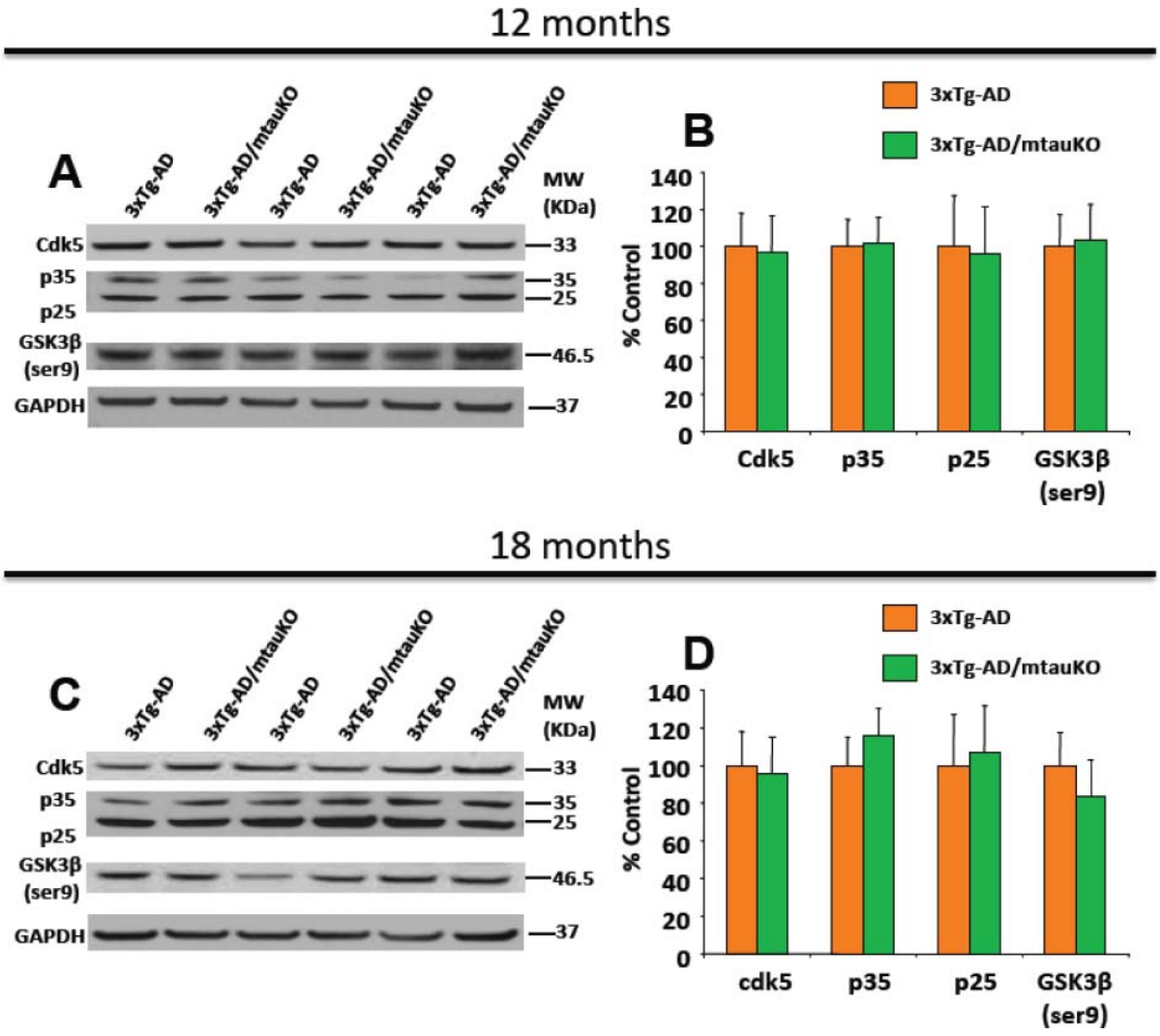

Figure 5

No differences in tau kinase activity (cdk5 and GSK3β) in 3xTg-AD and 3xTg- AD/mtauKO. (A, C) Immunoblot analysis of cdk5, p25/p35 and inactive GSK3β (phosphorylated at ser9) of protein extracts from whole-brain homogenates of 3xTg-AD and 3xTg-AD/mtauKO mice (n=8) at 12 and 18 months of age, shown as alternating lanes. (B, D) Quantification of A and C normalized to GAPDH and expressed as a percent of control shows no significant differences in the expression of cdk5, p35, p25 and GSK3β in the 3xTg-AD compared to 3xTg-AD/mtauKO at 12 (B) and 18 (D) months of age.

Similarly, the C-terminal fragments (CTFs) C83 and C99, which are membrane stubs produced by cleavage of APP by α-secretase and BACE1, respectively, were not altered between 3xTg-AD and 3xTgAD/mtauKO at 12 and 18 months (Figures 6A-D). The soluble and insoluble Aβ levels measured by sandwich ELISA were also unchanged between the 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months (Figures 7A-D). Consistent with the ELISA results, immunohistological analysis with the antibody 6E10 showed no difference in the intra/extra-cellular immunolabeling expression pattern of APP/Aβ in the 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months (Figure 7E). These results are consistent with previous studies showing that a reduction of tau (Roberson et al., 2007) does not influence Aβ pathology.

Discussion

Despite the abnormal hyperphosphorylation of tau and NFT accumulation observed in AD postmortem brains, this pathology is not genetically originated from tau mutations. New transgenic mouse models expressing wild-type tau were developed for tauopathies which displayed tau hyperphosphorylation. However, neurofibrillary tangles do not develop in these new models and most of these mice do not develop neuropathological changes associated with

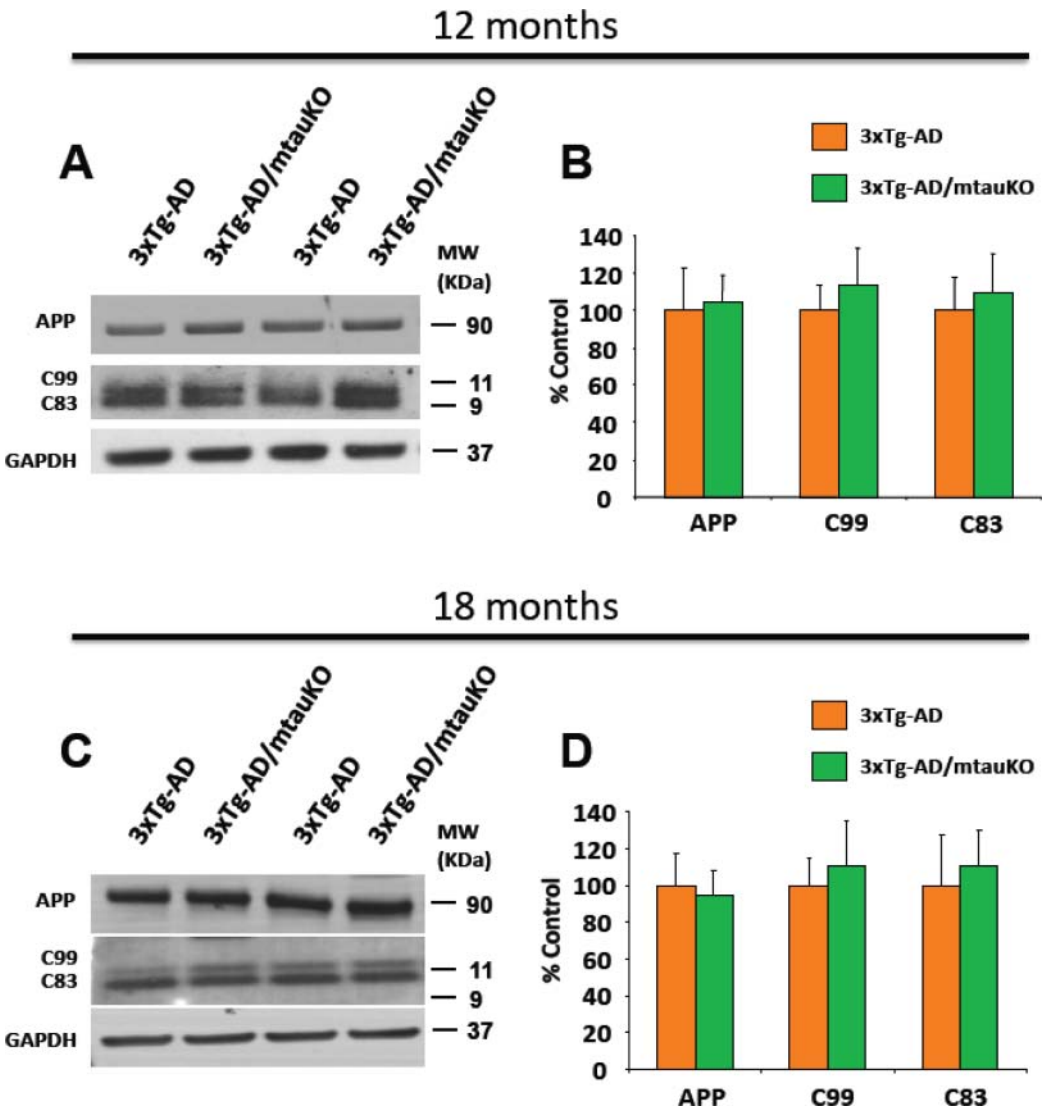

Figure 6

Deletion of mouse tau in the 3xTg-AD did not change APP processing. (A, C) Immunoblot analysis of APP holoprotein, C99 and C83 C-terminal APP fragment from whole- brain homogenates of 3xTg-AD and 3xTg-AD/mtauKO mice at 12 and 18 months of age, shown as alternating lanes. (B, D) Quantification of A and C normalized to GAPDH and expressed as a percent of control shows no differences in 3xTgAD compared to 3xTgAD/mtauKO mice.

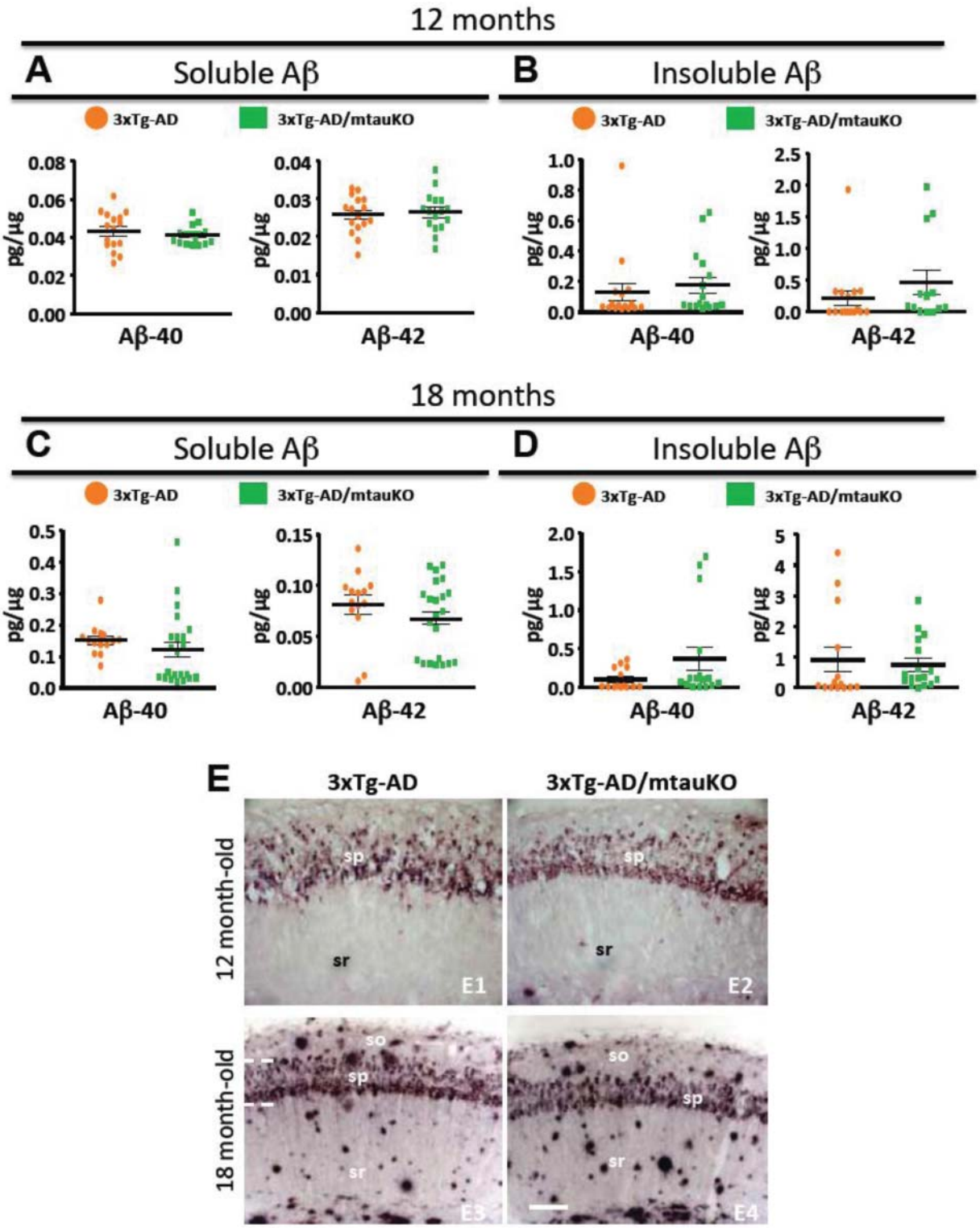

Figure 7

Deletion of mouse tau in the 3xTg-AD did not change Aβ pathology. (A, D) Brain Aβ measurements by sandwich ELISA of both the soluble (A and C) and insoluble (B and D), Aβ40 and Aβ42 fractions of 3xTg-AD and 3xTg-AD/mtauKO mice at 12 (A and B) and 18 (C and D) months of age revealed no significant differences in Aβ levels. (E) Light microscopic images immunostained with anti-Aβ antibody (6E10) in the hippocampus (CA1 areas) of 3xTg-AD (E1 and E3) and 3xTg-AD/mtauKO (E2 and E4) mice at 12 (E1 and E2) and 18 (E3 and E4) months of age (so: stratum oriens, sp: stratum pyramidale, sr: stratum radiatum). Scale bars: 100 µm.

the human tauopathies. Interestingly, genetically crossing mouse tau knockout mice with these human tau models showed a significant increase and acceleration of tau assembly (Andorfer et al., 2003; Ando et al., 2011). These studies suggest that murine tau hampers the aggregation pattern of human tau and formation of NFTs (Gotz et al., 1995; Brion et al., 1999; Ishihara et al., 1999; Probst et al., 2000; Duff et al., 2000; Ishihara et al., 2001; Higuchi et al., 2002). However, the hallmarks observed in postmortem AD brains include both extracellular Aβ-peptide plaque deposition and intracellular hyperphosphorylated microtubule-associated protein tau aggregation of NFTs (Querfurth and LaFerla, 2011). In this regard, critical studies have been carried out in transgenic mice suggesting modulatory interactions between Aβ and tau. Specifically, Hutton and collaborators showed that double mutant APP and tau transgenic mice developed enhanced neurofibrillary pathology compared with single mutant tau mice (Lewis et al., 2001), and Gotz *et al.* showed that intracranial Aβ fibril administration into mutant tau mice induced NFT formation in the amygdala (Gotz et al., 2001). In addition, we have previously determined that genetic manipulation and Aβ reduction by passive immunotherapy reduces the pre-existing tau pathology, indicating that Aβ can modulate the onset and progression of tau pathology by proteasome alterations (Oddo et al., 2004; Oddo et al., 2008; Tseng et al., 2008). Furthermore, other studies suggest that tau is a downstream target of Aβ that induces synaptic dysfunction and develops cognitive impairment in AD models (Roberson et al., 2007; Oddo et al., 2007).

To better understand the role of endogenous murine tau in the pathological events that occur in AD models and its interaction with Aβ-peptide, we developed a novel transgenic mouse model referred to as 3xTg-AD/mtauKO. Our results indicate that endogenous murine tau contributes to the progression of tau pathology in the 3xTg-AD mice with a considerable increase in PHF assembly and NFT formation. The high level of hyperphosphorylated tau observed in the 3xTg-AD may be related to the total amount of tau in both models. 3xTg-AD mice have both endogenous and human tau, while 3xTg-AD/mtauKO mice present only human tau. In this regard, it has been demonstrated that Aβ-amyloid is able to induce hyperphosphorylation of endogenous tau in transgenic mice referred to as Tg2576 (Otth et al., 2002). Therefore, it may be possible that the Aβ-amyloid in 3xTg-AD mice could trigger hyperphosphorylation of endogenous murine tau and contribute in exacerbating tau pathology in 3xTg-AD compared to 3xTg-AD/mtauKO mice. Recent studies, however, showed that murine tau could have anti-aggregation properties—due mainly to its N-terminal amino acid sequence (Ando et al., 2010)—and that crossing tau knockout mice with human tau models accelerated tau assembly (Andorfer et al., 2003; Ando et al., 2011). Other studies showed that the endogenous murine tau is capable of assembling PHFs and co-aggregating with human truncated tau (Chohan et al., 2005). Our data suggest that human tau in the 3xTg-AD mice may serve as a seeding platform to incorporate endogenous tau and propagate the pathology.

Recent interesting studies, however, suggest that NFTs may not be pathogenic, but rather could be a neuroprotective response to other disease stimuli such as oxidative stress or inflammation (Wittmann et al., 2001; Andorfer et al., 2005; Santacruz et al., 2005). Specifically, Ashe and her collaborators demonstrated in an inducible tau model that, after repression of tau expression, tau hyperphosphorylation and tangle formation progressed while memory function was recovered (Santacruz et al., 2005). Together with our findings, this suggests that endogenous tau may be involved in the progression of NFTs, yet it has a minimal influence on cognitive function in the 3xTg-AD mice. In addition, Davies' group demonstrated that NFT toxicity is not the only cause of the dramatic neuronal loss observed in mice expressing normal human tau (Andorfer et al., 2005).

Our study has significant implications in understanding the link between Aβ-amyloid and tau for the design of AD therapeutic strategies, as well as for the development of new models to better reproduce AD hallmarks.

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