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Loss of Tau Elicits Axonal Degeneration in a Mouse Model of AD

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Abstract

A central issue in the pathogenesis of tauopathy is the question of how tau protein dysfunction leads to neurodegeneration. We have previously demonstrated that the absence of tau protein is associated with destabilization of microtubules and impaired neurite outgrowth (Dawson et al., 2001, Rapoport et al., 2002). We now hypothesize that the absence of functional tau protein may render the central nervous system more vulnerable to secondary insults such as the overexpression of mutated beta amyloid precursor protein (APP) and traumatic brain injury. We therefore crossed tau knockout mice (Dawson et al., 2001) to mice overexpressing a mutated human APP (APP_{670,671}, A_{sw}) (Hsiao et al., 1996) and created a mouse model (A_{sw}/mTau^{-/-}) that provides evidence that the loss of tau causes degeneration of neuronal processes. The overexpression of APP_{670,671} in tau knockout mice, elicits the extensive formation of axonal spheroids. While spheroids are only found associated with Aβ plaques in mice expressing APP_{670,671} on an endogenous mouse tau background (Irizarry et al., 1997), A_{sw}/mTau^{-/-} mice have spheroids not only surrounding Aβ plaques but also in white matter tracts and in the neuropil. Plaque associated and neuropil dystrophic neurites and spheroids are prominent features of Alzheimer's disease (Masliah et al., 1993, Terry, 1996, Stokin et al., 2005). Thus our current data suggests that loss of tau may lead to neurodegeneration.

Keywords

Tau; APP; axonal degeneration; spheroids; head injury; immunization

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INTRODUCTION

The tauopathies represent a variety of progressive neurodegenerative diseases characterized by intracellular aggregates of the tau protein, which include frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease, and Alzheimer's disease (AD) (Goedert and Hasegawa, 1999, Sergeant et al., 1999, Buee et al., 2000, Reed et al., 2001, Togo and Dickson, 2002, Katsuse et al., 2003, Morris et al., 2003, Forman et al., 2004, Rademakers et al., 2004). Although these conditions are clinically diverse, the tauopathies tend to share several common features, including progressive dementia and extrapyramidal symptoms (Hernandez and Avila, 2007). Identifying the mechanisms of cellular dysfunction underlying tau pathology represents an important starting point for developing new therapeutic strategies.

Tau protein is a neuron-specific cytoskeletal protein that binds and stabilizes microtubules via the microtubule binding domain (Weingarten et al., 1975, Drubin et al., 1984, Binder et al., 1985, Caceres and Kosik, 1990, Ebner et al., 1998, Stamer et al., 2002). Although tau plays an important role in intracellular trafficking, its precise role in neurodegeneration remains an area of controversy. The FTDP-17 associated tau mutations are located predominantly in the microtubule binding region, indicating that this region is highly sensitive to disease-causing mutations (<http://www.alzforum.org/res/com/mut/tau/table1.asp>). Disruption of the microtubular network would affect a variety of intracellular processes such as intracellular transport, metabolism, mRNA trafficking, protein sorting and targeting (Buee et al., 2000). These, in turn, could have potential consequences for the maintenance of neuritic structures and synaptic plasticity.

Two major theories on the functionality of tau protein in neurodegeneration have been proposed, reviewed in (Buee et al., 2000). The first theory predicts that misfolded and aggregated tau protein causes a gain of toxic function by hindering normal axonal processes. This contention is supported by *in vitro* experiments demonstrating that mutations in the tau gene either decrease the binding of tau to microtubules or increase aggregation of the mutant tau proteins (Hasegawa et al., 1998, Hong et al., 1998, Dayanandan et al., 1999, Barghorn et al., 2000, Gambin et al., 2000). It is theorized that the decreased ability of mutated tau to bind to microtubules would allow for more free tau and therefore increase tau aggregation. The second theory predicts that neurodegeneration is due to the loss of tau caused either by a decrease in tau microtubule binding capabilities or by a decrease of the available pool of tau protein as a result of aggregation and/or phosphorylation.

Although a number of models have been generated to explore the gain of toxic tau function, the role of tau loss in neurodegeneration has not been explored as completely. Mouse tau knockout animals (Tau^{-/-} mice) (Dawson et al., 2001) generated in our laboratory, are an ideal model in which to study the effects of the loss of tau. We have previously demonstrated that the absence of tau in the neuronal cytoskeleton inhibits neuritic extension (Dawson et al., 2001) and destabilizes microtubules (Rapoport et al., 2002) although the Tau^{-/-} mice are otherwise healthy. However, since the onset of tau-related diseases often occur late in life or as a result of a co-existing insult, loss of tau may not have an immediate impact on the integrity of the microtubule system. Thus, exposure of affected neurons to additional insults may be required to elicit a diseased state.

Alzheimer's disease is one of the most common tauopathies and is characterized by intracellular tau and extracellular beta amyloid (A β) peptide accumulation. Patients with AD

present with neuronal degeneration, profound synaptic loss and the presence of a large numbers of amyloid plaques and dystrophic neurites (DNs). The A β peptide is derived through proteolytic processing of the amyloid precursor protein (APP) and mutations in APP that result in increased A β production have been shown to be causal in some types of familial AD. To determine whether loss of tau in the presence of amyloid deposition results in degeneration we crossed the tau knockout mouse Tau^{-/-} to transgenic mice overexpressing APP with the Swedish (sw) mutation (670/671_{KM→NL}) (A_{sw} mice) (Hsiao et al., 1996). Complete removal of tau in the A_{sw} mice elicited extensive degeneration of cortical and subcortical neurites, not normally observed in the A_{sw} mice alone, an increase in A β peptide, and more severe cognitive deficits. These results suggest that the loss of tau is one of the mechanisms of degeneration in tauopathies such as AD.

EXPERIMENTAL PROCEDURES

Animals

A_{sw} mice were crossed to Tau^{-/-} mice. Subsequently, A_{sw}/mTau^{+/-} mice were crossed to mTau^{+/-} mice to generate experimental mice A_{sw}/mTau^{-/-} and their littermate controls, A_{sw} mice, mTau^{-/-} mice, and non-transgenic WT mice. A_{sw}/mTau^{-/-}/hTau^{+/-} mice were generated by mating A_{sw} mice to mTau^{-/-}/hTau^{+/-} mice and then the mTau^{+/-}/hTau^{+/-} offspring were mated to A_{sw}/mTau^{+/-} mice from the A_{sw} mTau^{-/-} crosses described above. The original A_{sw} mice were on a C57B6/SJL background and the mTau^{-/-} and mTau^{-/-}/hTau^{+/-} mice were originally on a C57B6/SJL X C57B6/129Sv background.

Brain tissue harvesting

Mice were deeply anesthetized with ketamine (200 mg/kg), xylazine (10 mg/kg) and acepromazine (2 mg/kg). Then, mice were transcardially perfused with 30 ml phosphate-buffered saline. Brains were removed from the skull and divided into the right and left hemispheres; one hemisphere was frozen in liquid nitrogen for biochemical assays and the second hemisphere was fixed for 24 hours in 4% paraformaldehyde/phosphate buffered saline. The harvested hemisphere that had been frozen in liquid nitrogen was pulverized by a mortar and pestle over dry ice and divided into portions for use in protein and mRNA analysis. Sections were collected at a thickness of 40 μ m using a vibratome.

Immunohistochemistry

Brain sections were cut using a vibratome. A_{sw}/mTau^{-/-} mouse brains were highly susceptible to freezing damage when using the freezing microtome and therefore had to be sectioned on a vibratome. As a result, all mice were sectioned on the vibratome. For immunostaining, brain sections were first microwaved in SSC and antigen retrieval buffer (Vector Labs, Burlingame, CA) according to manufacturer's instructions. Then, tissue was incubated in 1% hydrogen peroxide, permeabilized by 0.1% Saponin and blocked with 10% goat serum. Primary antibodies were diluted to the final working concentration (see antibodies section) in 1% goat serum and then incubated overnight at 4°C. The Vectastain Elite ABC kit/DAB (diaminobenzidine) staining kit (Vector Labs, Burlingame, CA) was used according to manufacturer's instructions. Brain sections were dehydrated and mounted using DPX mounting media (Fluka, Milwaukee, WI, USA). Brain sections were mounted on slides, dehydrated, cleared and coverslipped using DPX mounting media (Fluka, Milwaukee, WI, USA). Brain sections were examined using a Nikon Eclipse TE200 microscope and images were recorded using a Nikon Digital Still Camera (DXM1200).

Antibodies

The following antibodies were used: human specific highly sensitive Tau13 (residues 9–18) (Garcia-Sierra et al., 2003) 1:33,000, (Tau13 was a kind gift from Dr. Lester Binder North Western University), human and mouse specific Tau5 (residues 210–236) (Abcam, Cambridge, MA., dilution 1:50), human specific antibody Tau14 (Zymed, San Francisco, CA), a pan polyclonal antibody specific for both mouse and human TAU17026 (a kind gift from Virginia Lee, University of Pennsylvania), synaptophysin (Dako, 1:300 dilution), 4G8 (center of A β , Covance, Princeton, NJ, dilution 1:2000), 6E10 (n-terminal of A β , Covance, Princeton, NJ, dilution 1:500), anti-Amyloid Precursor Protein, C-Terminal antibody (amino acids 751–770, Calbiochem, dilution 1:2000).

Histochemistry

The modified deOlmos cupric silver stain method (Wozniak et al. 1996, Holzman et al. 2000) was used to visualize spheroids and plaques. Fluoro-Jade B is a polyanionic fluorescein derivative which binds to degenerating neurons (Schmued and Hopkins, 2000). Sections were stained in a 0.001% Fluoro-Jade B (Histochem, Jefferson, AR, USA) solution prepared in 0.1% acetic acid for 30 minutes.

Axonal spheroid count

The brains of mice were sectioned sagittally (40 μ m) on a vibratome and stained using the de Olmos silver technique. The unbiased optical fractionator method (West et al., 1991; Long et al., 1998) was used to count the total number spheroids in a 20 x field spanning the genu of the corpus callosum and the cingulate cortex using the STEREO INVESTIGATOR 7.0 software (Microbrightfield, Williston, VT). This region was chosen because it is an easily identified region, which consistently displays high numbers of spheroids in the Asw/mTau–/– mice. This area was counted in a set of 6 systematic-random, evenly spaced sections beginning with the first sagittal slice obtained from the midline containing the hippocampus. The number of mice used for each experiment can be seen in Table 1. Group averages for control and experimental animals were generated and compared using the Student's t-test function (Prism GraphPad software).

Determination of plaque load

A set of 6 systematic-random 40 μ m sections per animal through the hippocampus and cortex were used to estimate amyloid load. Sections were stained with the de Olmos silver stain technique that visualizes both neuritic and non-neuritic plaques. Using the area fraction fractionator method STEREO INVESTIGATOR 7.0 software (Microbrightfield, Williston, VT) point grids were placed at random over the hippocampus and cortex and the number of points hitting plaque profiles were counted along with points hitting reference space. The product of the sum of points hitting each objects and the distance between points (the area per point) were used to find total area. The ratio of these areas will be expressed as the area fraction ($A_{\text{profiles}}/A_{\text{ref space}}$), which is proportional to volume fraction and represents the plaque load. The number of mice used for each experiment can be seen in Table 2.

Western blot analysis

Western blot analysis using various APP and tau antibodies was used to confirm the presence of the transgenic proteins and to semi-quantitatively determine the expression level of APP. As described in Dawson et al. (2001), frozen, pulverized mouse brain tissue was homogenized in cocktail containing protease inhibitors (Complete Mini, Roche, Indianapolis, IN). The amount of protein in each fraction was determined by the BCA Protein Assay (Pierce, Rockford, IL). The supernatants were mixed with Laemmli sample buffer, heated and separated by SDS-PAGE. Proteins were transferred onto a PVDF

membrane, which was then blocked in 5% non-fat dry milk, incubated overnight at 4degrees in primary antibody, washed then incubated for one hour in secondary antibody. The membrane was visualized using the ECL Plus kit (Amersham) sensitive to antigens in the picogram range.

The level of human APP expression in WT, A_{sw}, mTau^{-/-} and A_{sw}/mTau^{-/-} mice was determined semi-quantitatively by immunoblotting brain homogenate from a minimum of 3 mice per genotype and comparing it to a standard GAPDH a standard housekeeping protein. The intensity of staining was quantified by densitometry using the Kodak Image Station 440 CF and Kodak 1D image Analysis Software (Kodak, New Haven, CT).

Detection of Soluble and Insoluble A β

Soluble and insoluble pools of A₄₀ and A₄₂ were measured with a specific ELISA and differential brain extractions as described (Miao et al., 2005).

Behavioral testing

The Rotarod protocol adapted from Hamm et al. 1994 (Hamm et al., 1994) was used to assess motor coordination and balance (Crawley, 2000). The mice were first conditioned to the rotarod at a constant speed (16 rpm) for 60 seconds. This was done twice with a 5 minute interval between trials. For testing, mice were placed on the rotarod. The rotarod speed accelerated at a rate of 7. 2 rpm for 5 minutes starting at 4 rpm. Performance was assessed by measuring the latency time to either falling from the rod or turning 360 degrees twice (stationary and holding on to the rod). The Morris water maze tests the ability of the mice to locate a submerged platform. The trial ends if the mice cannot locate the platform in 1 minute. The mice performed 3 trials in a morning session and 3 trials in an afternoon session for 5 days. The mice were placed in varying quadrants of the platform for each trial but the location of the platform never varied. The radial arm water maze (Morgan et al., 2000, Gordon et al., 2001) measures learning and memory. The radial arm water maze tests the animal's ability to find a round (7. 5 cm diameter) submerged escape platform in a 105 cm diameter pool within a 60 sec interval. The pool is divided in to 6 arms (swim paths), each of which had aluminum sides with an open center in the middle of the pool. The platform is placed at the end of one arm. Spatial cues are present on walls of the testing room. The mice were given the opportunity to learn the location of the submerged platform in five consecutive acquisition trials. The number of errors the mouse made prior to locating the platform was recorded. An error constituted the entry in to a non-platform arm or entering the platform arm but leaving without climbing on to the platform. Mice making no decision in a 15 second period were assigned an error. The platform was placed in a different arm on each consecutive day and was always submerged. Twelve month old animals along with age-matched controls were pre-trained for 3 days on the radial arm water maze and tested on the morning of the fourth day. A probe trial was conducted on the fourth day in the afternoon without changing the location of the platform from the morning to measure retention memory. By the fourth day, non-transgenic and mTau^{-/-} mice consistently located the platform on the fourth trial. Since the measure of learning acquisition and memory retention was based on the number of errors it takes the mouse to find the platform in one minute, rather than the latency, slight motor deficits did not bias the results towards showing more memory deficits.

Traumatic Brain Injury (TBI)

Our murine TBI model (Lynch et al., 2002, Lynch et al., 2005) was adapted from a previously described model of closed cranial trauma for the rat (Marmarou et al., 1994). Twenty four to twenty six-week-old mice were anesthetized with general anesthesia and positioned in a stereotactic device and the skull exposed. A concave 3-mm metallic disc was

glued to the skull immediately caudal to bregma. A 2.0 mm diameter pneumatic impactor (Air-Power, Inc. High Point, NC, USA), discharged at 6.8 ± 0.2 m/s with a head displacement of 3 mm, was used to deliver a single midline impact to the disc surface. The scalp was sutured and the animals were allowed to recover. The animals were sacrificed when they reached forty to forty-two weeks of age.

Immunization

The mice were be immunized as previously described (Schenk et al., 1999, Janus et al., 2000, Morgan et al., 2000). Briefly, lyophilized A β 1–42 peptide (Bachem) was suspended in deionized water and 10 $\times$ PBS is added to the mixture to obtain a 2 mg/ml, 1 $\times$ PBS (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) solution. The suspension was incubated overnight at 37 °C and used on that day. KLH for control injections was prepared in the same manner (Morgan et al., 2000). Mice were immunized with 100 μ g A β 1–42 mixed 1:1 with Freund's complete adjuvant for the first set of injections at 6 weeks of age. A boost of A β 1–42 (freshly prepared) was administered mixed with 1:1 Freund's incomplete adjuvant at 8 weeks of age followed by three monthly injections. A β 1–42 in PBS only was administered from the sixth through the eleventh month. Mice were tested in the radial-arm water maze as described above weeks following the final vaccination. Immediately following the radial-arm water maze testing mice were sacrificed and tissue was harvested as described above. Serum was collected at sacrifice and used to measure antibody titers as described previously (Morgan et al., 2000).

RESULTS

In order to explore the loss of tau in neuronal degeneration, we examined the concurrent effects of tau deficiency (mTau^{-/-}) (Dawson et al., 2001) and expression of the mutated APP protein. By mating mTau^{-/-} mice to the A_{sw} mice that overexpress the human amyloid precursor protein carrying the Swedish mutation 670/671_{KM→NL} (Hsiao et al., 1996), we generated mice that overexpress an Alzheimer's disease APP protein on a tau null background (A_{sw}/mTau^{-/-} mice).

The Loss of Tau in A_{sw} Mice Elicits Profound Neurite Dystrophy

Brain slices from 12 month old A_{sw}, mTau^{-/-} and A_{sw}/mTau^{-/-} mice were assessed by the de Olmos silver stain (DeOlmos and Ingram, 1971, Dikranian et al., 2001), a cupric silver stain technique that is routinely used to detect neuronal degeneration and visualize plaques (Wozniak et al., 1996, Holtzman et al., 2000). Both the A_{sw}/mTau^{-/-} and the A_{sw} mice had A β plaques with and without associated dystrophic neurites. However, only the A_{sw}/mTau^{-/-} mouse brains had extensive spheroids that were not associated with plaques (Figures 1 and 2). Axonal spheroids have been previously reported in the several mouse models that overexpress mutated APP (Stokin et al., 2005, Wirths et al., 2006, Wirths et al., 2008) and in patients with Alzheimer's disease (Dai et al., 2002, Stokin et al., 2005). The spheroids were most abundant in the anterior cingulum, cingulate cortex, and in the regions surrounding the genu of the corpus callosum (Figure 1C), subiculum (Figure 1F), and anterior thalamic nuclei (Figure 1L). Moderate amounts of spheroids were also found in the substantia nigra, anterior amygdaloid area and the ventrolateral orbital cortex as well as in the basal nucleus of Meynert (data not shown). Low numbers of spheroids were found throughout the spinal cord, brain stem and the rest of the cortex. In contrast to the abundance of spheroids in the A_{sw}/mTau^{-/-} mice, only an occasional DN was found throughout the brains of single transgenic A_{sw} and mTau^{-/-} mice (Figure 1A–C and Figure 1B–H respectively). Higher magnification images of spheroids and plaques are presented in Figure 2.

We quantified the number of plaque free spheroids in the A_{sw} , $mTau^{-/-}$ and $A_{sw}/mTau^{-/-}$ mice at two time points, 10 months and 12 months (Table 1; Figure 3), as development of plaques in APP transgenic mice is time dependent. Animals display almost no plaques at 10 months while at 12 months all animals display plaques (see text below and Figure 7A and B). At ten months the absence of tau in the $A_{sw}/mTau^{-/-}$ mice resulted in a modest increase in the number of spheroids when compared to the endogenous mouse tau expressing controls A_{sw} mice, $p=0.0461$ (Figure 3). However, at the same age, an equal number of spheroids was seen in the $A_{sw}/mTau^{-/-}$ mice as in the single transgenic $mTau^{-/-}$ control mice, $p=0.418$ (Table 1; Figure 3) indicating that spheroids at this age are most likely due to the loss of tau and not mutated APP expression. We observed a dramatic increase in the number of spheroids between 10 and 12 month old $A_{sw}/mTau^{-/-}$ mice, $p<0.0001$ (Table 1, Figure 3). It is important to note that the plaque load also increased between the ages of 10 and 12 months (Table 2, Figure 7). The increase in spheroids is dependent on both the overexpression of mutated APP and the loss of tau, since neither A_{sw} nor $mTau^{-/-}$ single transgenic mice showed an increase in the number of spheroids between the ages of 10 and 12 months (Table 1, Figure 3). At 12 months, the $A_{sw}/mTau^{-/-}$ mice had over a 30 fold increase in spheroids compared to A_{sw} mice, $p<0.0001$, and approximately a 5 fold increase in spheroids compared to $mTau^{-/-}$ mice, $p<0.0001$ (Table 1, Figure 3). This data implies that, although the loss of tau alone is sufficient to cause neuritic abnormalities, the overexpression of mutated APP acts synergistically to increase this pathology.

A subset of spheroids are axonal swellings

The high number of spheroids varying in size from 1 to 10 μm found predominantly in axonal tracts indicates that some of the spheroids most likely represent axonal swellings. Similar axonal spheroids are prominent components in many neuropathies. Ultrastructural electron microscopy analysis revealed the spheroids were structures filled with cytoskeletal debris, degenerating mitochondria, vesicular and membranous multilamellar bodies and vacuoles (Figure 4). Furthermore, staining with FluoroJadeB (Figure 5) a stain which is specific for degenerating neurons and neuronal processes, suggested that the spheroids were most likely of neuronal origin. Immunocytochemistry was used to further confirm the identity of the spheroids. A subset of the spheroids was positive with a synaptophysin antibody (Figure 6A and B) an antibody specific for axons and pre-synaptic terminals.

The loss of tau decreased the number of neuritic plaques

In order to assess the effect of the absence of tau on cortical and hippocampal plaque burden the percentage of total area covered by plaques was determined using unbiased stereology. As expected, there was a trend towards an increase in both neuritic (associated with dystrophic neurites) and non-neuritic plaques in both A_{sw} and $A_{sw}/mTau^{-/-}$ mice with age (10 to 12 months) (Table 2, Figure 7); the fact that this trend did not reach statistical significance between 10 and 12 month old A_{sw} mice was most likely due to the large variability in the plaque load between animals, which is typical for mutant APP models. Furthermore, the absence of tau protein caused a significant decrease in the percentage of total area covered by neuritic plaques in the $A_{sw}/mTau^{-/-}$ mice when compared to the A_{sw} mice at both 10 ($p=0.037$) and 12 ($p=0.050$) months of age (Table 2, Figure 7A). There was no significant difference in the area of non-neuritic plaques between $A_{sw}/mTau^{-/-}$ and A_{sw} mice (Table 2, Figure 7B).

The decrease in neuritic plaques in the $A_{sw}/mTau^{-/-}$ mice compared to the A_{sw} mice was unexpected and we subsequently assessed the levels of $A\beta$ in the A_{sw} and $A_{sw}/mTau^{-/-}$ mice using an $A\beta$ ELISA. Surprisingly, all forms of $A\beta$ (40 and 42, soluble and insoluble) were increased in $A_{sw}/mTau^{-/-}$ mice when compared to A_{sw} mice (Table 3, Figure 7D). The total level of $A\beta$ rose to 4571 ± 184 pg/mg of total protein in the $A_{sw}/mTau^{-/-}$ mice, a 6.9

fold increase over the amount of total A β in A_{sw} mice, 662 $\pm$ 105 pg/mg. This increase was not due to an increase in overall APP expression as measured by semi-quantitative analysis of Western blots probed with the N-terminal 6E10 and carboxy-terminal APP-C antibodies (Figure 7E and D). Western blot analysis was used to confirm the expression of the various transgenes (Figure 7C). Immunostaining of brain slices with 4G8, a monoclonal antibody specific to amino acids in the A β peptide portion of APP, stained plaques in brain slices of both A_{sw} and A_{sw}/mTau^{-/-} mice. Significantly 4G8 also immunostained neurons in the CA1 region of the hippocampus, the subiculum and in layer 5 of the cortex in A_{sw}/mTau^{-/-} mice (Figure 8A-C). Diffuse and punctate staining was evident as previously reported in APP overexpressing models of AD (Wirhth et al., 2004, Gomez-Ramos and Asuncion Moran, 2007, LaFerla et al., 2007).

When transgenic mice are generated, the potential exists that chromosomal disruptions may occur. To determine that the spheroid phenotype was due to the expression of the APP_{670,671} protein and the loss of tau interactions rather than non-specific DNA disruption in the mTau^{-/-} mouse, we reconstituted tau protein in the A_{sw}/mTau^{-/-} mice by mating them to transgenic mice that express human tau protein from the entire human tau gene (hTau^{+/-}/mTau^{-/-} mice) (Dawson et al., 2001). The hTau^{+/-}/mTau^{-/-} mice are on a mouse tau knockout background and express all six human tau mRNA and protein isoforms at approximately three times the level of endogenous mouse tau. At 12 months of age the A_{sw}/mTau^{-/-}/hTau^{+/-} mice have significantly less spheroids than the A_{sw}/mTau^{-/-} mice, $p < 0.0001$ (Table 1, Figure 3). As expected, the spheroid pathology in the brains of A_{sw}/mTau^{-/-}/hTau^{+/-} mice at 10 and 12 months did not significantly differ from the A_{sw} mice, $p = 0.462$ (Table 1, Figure 3). These results confirm that the extensive axonal degeneration observed in the A_{sw}/mTau^{-/-} mice is a result of the loss of tau in the presence of mutated APP_{670,671} protein.

A_{sw}/mTau^{-/-} mice have vestibulomotor and cognitive deficits

We next assessed whether the observed histological abnormalities were associated with functional deficits. Vestibulomotor function was determined by the Rotarod test (Figure 9C). There was no significant decrease in Rotarod latency between WT and single transgenic mTau^{-/-} and A_{sw} mice (Figure 9C). However, the A_{sw}/mTau^{-/-} mice had a significant decrease in Rotarod performance when compared to WT and single transgenic mouse controls (mTau^{-/-} and A_{sw} mice). These results suggest that the deletion of tau protein in conjunction with mutated APP overexpression has detrimental effects on motor function.

The Morris water maze has been previously used to test reference memory in A_{sw} mice, and at 12 months of age A_{sw} mice generally do not show differences in performance when compared to WT mice (reviewed in Kobayashi and Chen (Kobayashi and Chen, 2005)). Figure 9 shows that 12 month old A_{sw}/mTau^{-/-} mice were significantly impaired in their ability to locate the platform. While the age matched A_{sw}, non-transgenic (WT) and mTau^{-/-} mice were able to locate the submerged platform within 20 seconds on day 5 of training, the A_{sw}/mTau^{-/-} mice never learned to locate the platform ($p < 0.0001$ for all of the ANOVA analysis comparing the A_{sw}/mTau^{-/-} mice to each of the other control groups in Figure 9A).

Because of the deficits in motor function identified by the Rotarod test in the A_{sw}/mTau^{-/-} mice, the concern arose that the results of the Morris Watermaze may have been influenced by impairment of swimming ability. We therefore performed the radial arm water maze task to verify abnormalities in spatial learning and memory (Gordon et al., 2001, Dawson et al., 2007). Learning and memory in this task is measured by the number of errors an animal makes locating a submerged platform instead of the length of time it takes to find a submerged platform, therefore, any motor deficits affecting the rate of swimming will not

bias the results towards an increased cognitive deficit but instead may actually underestimate the number of errors due to slower swimming.

Consistent with the Morris watermaze results, the $A_{sw}/mTau^{-/-}$ mice performed significantly worse than the WT ($p=0.003$) and $mTau^{-/-}$ ($p=0.025$) controls (Figure 9B). Contrary to the data obtained from the Morris water maze, the A_{sw} mice also performed worse than WT ($p\leq 0.0001$) and $mTau^{-/-}$ ($p<0.001$) controls. This is consistent with the fact that the radial arm water maze (RAWM) protocol allows the platform to remain in the same location for each day's trials; however, the platform location is changed each day making it more difficult and therefore more sensitive to test for spatial working memory. Overall, there was no significant difference between the A_{sw} mice and $A_{sw}/mTau^{-/-}$ mice ($p=0.303$). But because the RAWM protocol requires mice to learn the rule that the platform is in the same arm on each trial within a day, but in a different arm each day we were able to determine that $A_{sw}/mTau^{-/-}$ mice are deficient in their working spatial memory. While the WT ($p=0.0064$), $mTau^{-/-}$ ($p=0.0002$), and A_{sw} ($p=0.042$) mice improved in the task between day 1 and day 4 of training, the $A_{sw}/mTau^{-/-}$ mice did not ($p=0.718$) (Figure 9C). Following the day 4 trials, retention memory for the platform location was tested 3 hours past the last test trial. Both the A_{sw} ($p=0.342$) and $A_{sw}/mTau^{-/-}$ ($p=0.106$) mice were deficient in locating the platform in this task (difference between day 4 and the probe trial, Figure 9C).

Head Injury Accelerates Axonal Degeneration in $A_{sw}/mTau^{-/-}$ Mice

From the above data, it is clear that in the complete absence of tau function, neurons stressed with the expression of mutated APP_{670,671} develop severe axonopathy. We next explored whether this pathology was uniquely due to the absence of tau function and overexpression of APP_{670,671} or whether other stressors such as traumatic brain injury could trigger axonal degeneration in the $mTau^{-/-}$ mice. Traumatic brain injury is a clinically relevant factor in exacerbating tau pathology (Geddes et al., 1996, Geddes et al., 1999), as head injury has been proposed as a predisposing factor for several tauopathies such as chronic traumatic encephalopathy (reviewed in (Jordan, 2000) and Alzheimer's disease (Heyman et al., 1984, Mortimer et al., 1985, Guo et al., 2000, Jordan, 2000, Plassman et al., 2000). We therefore decided to challenge mice lacking tau function with or without the overexpression of APP_{670,671} ($A_{sw}/Tau^{-/-}$ and $Tau^{-/-}$ mice) with traumatic brain injury (TBI). A_{sw} mice were used as controls. Mice were injured with TBI between the ages of 6 to 7 months and sacrificed at 10 months, a time period when very few spheroids or A β plaques are present in uninjured mice.

There was no significant increase in plaque load post TBI in either the A_{sw} or the $A_{sw}/mTau^{-/-}$ mice when compared to their non-injured controls (Table 2). However, a significant increase in axonal spheroids was seen in the cingulum, cingular cortex, thalamus and subiculum of TBI $A_{sw}/mTau^{-/-}$ mice when compared to age matched non-injured $A_{sw}/mTau^{-/-}$ controls, $p=0.0014$ (Table 1, Figure 10). No significant increase in spheroids was seen post TBI A_{sw} mice, $mTau^{-/-}$ mice or the $A_{sw}/mTau^{-/-}$ mice reconstituted with the human tau protein, $A_{sw}/mTau^{-/-}/hTau^{+/-}$ mice, when compared to their age matched non-injured controls (Table 1, Figure 10). Notably, the $A_{sw}/mTau^{-/-}$ mice had over four times as many spheroids as either the A_{sw} , $mTau^{-/-}$ or the $A_{sw}/mTau^{-/-}/hTau^{+/-}$ mice, $p=0.0039$, $p=0.001$ and $p=0.005$ respectively, indicating that the loss of tau and the expression of APP_{670,671} are both required for the extensive axonopathy seen in the $A_{sw}/mTau^{-/-}$ mice post TBI.

Immunization with the A β Peptide Does Not Ameliorate Axonal Degeneration in A_{sw}/mTau^{-/-} mice

Several recent studies have demonstrated that the removal of A β from mouse brain overexpressing mutated APP protein improves neurodegeneration and behavioral deficits (Janus et al., 2000, Morgan et al., 2000). Both active (Schenk et al., 1999, Morgan et al., 2000) and passive (Bard et al., 2000, Wilcock et al., 2004) immunization have been used with positive results. We therefore actively immunized our A_{sw} and A_{sw}/mTau^{-/-} mice on a monthly basis with fibrillar A β peptide starting at 6 weeks of age through 11 months. The mice were tested for learning, memory and motor function and assessed for the number of spheroids and for plaque load at 12 months (Figure 11). All of the A β immunized mice showed positive titers against the A β peptide (A β immunized mice, 700 to 6,200 μ g of A β antibody per ml of serum compared to control A_{sw} mice, 24 to 120 μ g of A β antibody per ml of serum). The anti-A β antibody titer did not correlate with the individual plaque loads.

Both the neuritic and non-neuritic plaque loads in the A_{sw} mice decreased with A β immunization (Figure 11A) as previously reported by (Schenk et al., 1999, Morgan et al., 2000). In the A_{sw}/mTau^{-/-} mice, only the non-neuritic plaque load decreased significantly while there was no significant change in the neuritic plaque load (Figure 11A). Nevertheless, no significant decrease in the number of spheroids was seen in either the A_{sw} (data not shown) or in the A_{sw}/mTau^{-/-} mice (Figure 11B). The decrease in plaques did correlate with cognitive deficits in the A_{sw} mice; A β immunization improved the performance on the radial arm water maze (ANOVA, $p=0.004$) (Figure 11C). However, no improvement was seen in the A_{sw}/mTau^{-/-} mice treated with A β (Figure 11D). Immunization with A β had no effect on Rotarod results in either the A_{sw} or A_{sw}/mTau^{-/-} mice (data not shown).

Because immunization with A β did not correlate with improvement in axonal pathology or functional outcomes in the A_{sw}/mTau^{-/-} mice, we quantified A β levels by ELISA on A β immunized A_{sw} and A_{sw}/mTau^{-/-} mice (Table 3, Figure 11E). Significant decreases in A β occurred in both soluble and insoluble 40 A β and soluble 42 A β . The insoluble 42 A β fraction in A_{sw}/mTau^{-/-} mice demonstrated a trend in A β reduction between immunized versus non immunized mice, but failed to reach statistical significance. However, there was a significant decrease in total A β in both mouse lines; 81% in A_{sw} mice and 76% in A_{sw}/mTau^{-/-} mice.

DISCUSSION

In the current study, we present evidence that the loss of tau results in neurodegeneration and cognitive deficits. By 12 months of age, tau knockout mice stressed with the overexpression of mutated human APP developed an age-dependent severe increase in axonal spheroids compared to mice that overexpress mutated human APP on a mouse wild type tau background. As a result, the A_{sw}/mTau^{-/-} mice have increased cognitive and motor deficits that are not improved by A β immunization. Furthermore, traumatic brain injury hastens the onset of pathology in tau deficient animals. These results suggest that the loss of tau may predispose to axonal pathology and neurodegeneration in the stressed central nervous system.

Interestingly, we find that spheroids correlate with behavioral deficits in A_{sw}/mTau^{-/-} mice to a much greater extent than plaque load or A β brain levels. While the immunization of A_{sw}/mTau^{-/-} mice with A β reduced non-neuritic plaque burden and total brain A β , the neuritic plaque burden and spheroid pathology remained unchanged, and behavioral outcomes did not improve. This is consistent with data published by Stokin et al., 2008 (Stokin et al., 2008) and suggests APP-induced axonal defects are not caused by A β . As

previously reported in A_{sw} mice, $A\beta$ immunization was associated with reduced total plaque burden, reduced levels of brain $A\beta$ and improved cognitive outcomes.

Although tau aggregation and $A\beta$ deposition are both associated with the pathology of AD, it remains a source of debate as to whether amyloid deposition in plaques is the primary cause of cell death. Our data are consistent with recent clinical trials which failed to show functional improvement following $A\beta$ immunization. A six-year follow-up of eight patients from the AN1792 trial confirmed that as expected vaccination with full-length $A\beta_{42}$ can clear amyloid plaques (Holmes et al., 2008). However in spite of the plaque decrease, evidence was presented that this clearance did not slow disease progress and in fact, several of those who had near complete plaque removal at autopsy had clinically deteriorated to severe dementia (Holmes et al., 2008). Because most models of AD, including the A_{sw} mice, display limited AD associated pathology, a second hit is required to display the neuritic degeneration that occurs in AD. These results are consistent with the hypothesis that loss of normal tau may also play a primary role in initiating the cascade of neuronal injury in clinical disease.

Axonal dystrophy and spheroids are a prominent component in many neuropathies including those due to metabolic disease, toxic exposures, nutritional deficiencies, inflammatory disorders, and ischemic disease (Takahashi et al., 1997, Griffiths et al., 1998, Dewar et al., 1999, Probst et al., 2000, Raff et al., 2002). Histologically, dystrophic neurites correspond to axonal swellings, which often contain abnormal accumulations of axonal cargos and cytoskeletal filaments (Masliah et al., 1993). Recently Stokin et al (2005) (Stokin et al., 2005) identified axonal defects in the form of large axonal swellings in brains of AD patients and in the brains from A_{sw} mouse model of AD. Similar to our findings and other reports describing dystrophic neurites (Masliah et al., 1996, Brendza et al., 2003a, Brendza et al., 2003b, Wirths et al., 2006), these swellings consisted of vesicles, mitochondria, multilamellar bodies and vacuoles. Interestingly, Stokin et al. (Stokin et al., 2008) also reported that the formation of axonal swellings was exacerbated by reduction in kinesin-1. This data implicated a potentially causative role of axonal transport deficits in AD. Several studies have shown that tau protein may function in the regulation of loading and unloading cargos on to microtubules (Ebneth et al., 1998, Stamer et al., 2002, Mandelkow et al., 2003, Vershinin et al., 2007, Dixit et al., 2008, Vershinin et al., 2008). An increase of local tau concentration promotes cargo detachment from microtubules, while a decrease in tau concentration allows cargos to remain on microtubular tracts. These data suggest that a high concentration of tau would be required at points along the axons where cargo needs to detach from microtubules such as branch points and at synaptic terminals. In fact, several publications have shown that the concentration of tau protein is highest at the junction of the distal end of an axon and the growth cone (Black et al., 1996, Kempf et al., 1996). It has also been reported that concentrations of tau isoforms vary at growth tips and branch points (Kosaka et al., 2004). It would therefore be predicted that a decrease or absence of tau protein in these regions of the axon could result in the accumulation of cargo such as vesicles, mitochondria and other organelles. The areas with the highest numbers of axonal spheroids in the $A_{sw}/mTau^{-/-}$ mice include the circuit of Papez, which involves the cingulate cortex, cingulum, subiculum and thalamus. Impairment of this pathway has been implicated in AD, and would be expected to disturb the flow of information through the Papez circuit and the perforant path (Jones, 1993, Villain et al., 2008).

Although neuritic plaques are decreased, $A_{sw}/mTau^{-/-}$ mice have approximately a seven fold increase in $A\beta$ in their brains when compared to A_{sw} mice. This increase is not the result of increased APP expression. The increase in $A\beta$ appears to result in neuronal accumulation which is evident in the $A_{sw}/mTau^{-/-}$ mice but not in A_{sw} mice. Intracellular accumulation of $A\beta$ is observed in both AD patients and in animal models of AD and is

believed to be one of the early events in the pathogenesis of Alzheimer's disease (Wirh's et al., 2004, Gomez-Ramos and Asuncion Moran, 2007, LaFerla et al., 2007). The intracellular accumulation of A β and/or APP is consistent with defects in axonal transport. APP vesicles are transported on microtubules by fast axonal transport and any defects in transport would likely cause accumulation in the soma of affected neurons. A β immunization may be more efficient in clearing parenchymal as opposed to intracellular A β . Therefore, the intracellular instead of extracellular accumulated APP fragments that are immunogenic with an antibody to the A β peptide may explain the lack of pathological and behavioral improvement with A β immunization in the A_{sw}/mTau^{-/-} mice.

Interestingly, a recent paper by Roberson et al. 2007 reported that the reduction of endogenous tau ameliorates A β -induced deficits. This model utilized our tau knockout mice crossed to the J20 mouse line that expresses both the Swedish (670/671_{KM→NL}) and the Indiana (717_{V→F}) AD APP mutations under the regulation of the platelet-derived growth factor promoter (Roberson et al., 2007). In contrast to our current data, which suggests that a loss of tau is associated with increased mortality and functional impairment, in this model, the reduction of tau protein was associated with an improvement in the rate of survival and in cognition. While axonal degeneration was not assessed in the Roberson APP/mTau^{-/-} mouse model, a decrease in hyperactivity and susceptibility to excitotoxin-induced seizures was reported when one or two tau alleles were knocked out. It is important to note that the removal of tau protein also reduced susceptibility to excitotoxin-induced seizures in mice not transgenic for the human APP protein. However, since the publication of this study attributing tau reduction to A β toxicity (Roberson et al., 2007), two preliminary reports (Roberson et al., 2008 Neuroscience meeting; Ittner et al., 9th International conference on AD/PD 2009) and a review paper (Palop and Mucke, 2009) have suggested that the improvement in premature mortality and behavioral deficits seen in the hAPP mouse models may have resulted from a decrease in epileptiform activity. These conclusions are further supported by findings that a widely used anticonvulsant, valproic acid, also decreases A β toxicity in a hAPP transgenic mouse model (Qing et al., 2008). Therefore, the resultant improvement in survival and behavioral deficits in these mice may result from the dramatic suppression of epileptiform activity which in turn may mask the deficits that would normally occur from the interaction of mutated APP overexpression and the absence of tau. While seizures may occur in a small percentage of AD patients, they are not considered a cardinal feature of this disease (Amatniek et al., 2006).

Furthermore, the disparities between our two models may be due to the timing of axonopathy. The Morris water maze in the Roberson model was performed between 4–7 months, a time when neuritic plaques are not present in the J20 mice. In contrast, our mice were tested at 12 months when a significant proportion of the plaques are neuritic. While it is debated whether plaque formation is the primary entity that causes AD, the formation of neuritic plaques may be a good indication of the neuritic pathology throughout the brain. Therefore, while the signs of neurodegeneration reported in the Roberson paper may be due to the overexpression of APP and increased epileptic activity as has been previously proposed, the increased death and cognitive impairment seen in our model may be the result of axonal dystrophy which occurs at a later age.

In summary, we demonstrate that the loss of tau exacerbates neuronal injury in a model of mutant APP overexpression. In particular, the absence of tau function in animals that overexpress APP results in axonal pathology and neuritic spheroids in the medial forebrain and other areas typically affected by AD. These findings are consistent with a prior report in which the loss or severe reduction of tau protein characterized several cases of sporadic frontal temporal dementia (Zhukareva et al., 2001) Unlike animals that overexpress APP but have normal tau function, A_{sw}/mTau^{-/-} mice do not demonstrate functional improvement

after A β immunization, and have accelerated histological and functional deficits following other forms of CNS stress, such as closed head injury. A more complete understanding of the role of aberrant tau function in the setting of A β deposition or other stressors to the central nervous system may guide future therapeutic approaches for the neurological diseases characterized by tauopathy.

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Abbreviations

Aβ	beta amyloid peptide
AD	Alzheimer's disease
APP	amyloid precursor protein
APP_{670,671}	APP with the Swedish (sw) mutation (670/671 _{KM→NL})
A_{sw}	transgenic mice expressing mutated APP _{670,671}
A_{sw}/mTau^{-/-}	hemizygous A _{sw} mice on a homozygous tau knockout background
A_{sw}/mTau^{-/-}/ hTau^{+/-}	triple transgenic mice hemizygous for mutated APP _{670,671} and the human tau gene on a homozygous mouse tau knockout background
CBD	corticobasal degeneration
DNs	dystrophic neurites
FTDP-17	frontotemporal dementia and Parkinsonism linked to chromosome 17
hTau^{+/-}	whole human tau gene hemizygous transgenic mice
mTau^{-/-}	mouse tau knockout mice
mTau^{-/-}/hTau^{+/-}	whole human tau gene hemizygous transgenic mice on a mouse tau knockout background
PSP	progressive supranuclear palsy
TBI	traumatic brain injury
WT	non-transgenic mice

References

- Amatniek JC, Hauser WA, DelCastillo-Castaneda C, Jacobs DM, Marder K, Bell K, Albert M, Brandt J, Stern Y. Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia*. 2006; 47:867–872. [PubMed: 16686651]
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med*. 2000; 6:916–919. [PubMed: 10932230]

- Barghorn S, Zheng-Fischhofer Q, Ackmann M, Biernat J, von Bergen M, Mandelkow EM, Mandelkow E. Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias. *Biochemistry (Mosc)*. 2000; 39:11714–11721.
- Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol*. 1985; 101:1371–1378. [PubMed: 3930508]
- Black MM, Slaughter T, Moshiah S, Obrocka M, Fischer I. Tau is enriched on dynamic microtubules in the distal region of growing axons. *J Neurosci*. 1996; 16:3601–3619. [PubMed: 8642405]
- Brendza RP, O'Brien C, Simmons K, McKeel DW, Bales KR, Paul SM, Olney JW, Sanes JR, Holtzman DM. PDAPP; YFP double transgenic mice: a tool to study amyloid-beta associated changes in axonal, dendritic, and synaptic structures. *J Comp Neurol*. 2003a; 456:375–383. [PubMed: 12532409]
- Brendza RP, Simmons K, Bales KR, Paul SM, Goldberg MP, Holtzman DM. Use of YFP to study amyloid-beta associated neurite alterations in live brain slices. *Neurobiol Aging*. 2003b; 24:1071–1077. [PubMed: 14643378]
- Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. 2000; 33:95–130.
- Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature*. 1990; 343:461–463. [PubMed: 2105469]
- Crawley, JN. What's wrong with my mouse: Behavioral phenotyping of transgenic and knockout mouse. New York: John Wiley and Sons; 2000.
- Dai J, Buijs RM, Kamphorst W, Swaab DF. Impaired axonal transport of cortical neurons in Alzheimer's disease is associated with neuropathological changes. *Brain Res*. 2002; 948:138–144. [PubMed: 12383965]
- Dawson HN, Cantillana V, Chen L, Vitek MP. The tau N279K exon 10 splicing mutation recapitulates frontotemporal dementia and parkinsonism linked to chromosome 17 tauopathy in a mouse model. *J Neurosci*. 2007; 27:9155–9168. [PubMed: 17715352]
- Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci*. 2001; 114:1179–1187. [PubMed: 11228161]
- Dayanandan R, Van Slegtenhorst M, Mack TG, Ko L, Yen SH, Leroy K, Brion JP, Anderton BH, Hutton M, Lovestone S. Mutations in tau reduce its microtubule binding properties in intact cells and affect its phosphorylation. *FEBS Lett*. 1999; 446:228–232. [PubMed: 10100846]
- DeOlmos JS, Ingram WR. An improved cupric-silver method for impregnation of axonal and terminal degeneration. *Brain Res*. 1971; 33:523–529. [PubMed: 4109198]
- Dewar D, Yam P, McCulloch J. Drug development for stroke: importance of protecting cerebral white matter. *Eur J Pharmacol*. 1999; 375:41–50. [PubMed: 10443563]
- Dikranian K, Ishimaru MJ, Tenkova T, Labruyere J, Qin YQ, Ikonomidou C, Olney JW. Apoptosis in the in vivo mammalian forebrain. *Neurobiol Dis*. 2001; 8:359–379. [PubMed: 11447994]
- Dixit R, Ross JL, Goldman YE, Holzbaur EL. Differential regulation of dynein and kinesin motor proteins by tau. *Science*. 2008; 319:1086–1089. [PubMed: 18202255]
- Drubin DG, Caput D, Kirschner MW. Studies on the expression of the microtubule-associated protein, tau, during mouse brain development, with newly isolated complementary DNA probes. *J Cell Biol*. 1984; 98:1090–1097. [PubMed: 6421824]
- Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol*. 1998; 143:777–794. [PubMed: 9813097]
- Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *NatMed*. 2004; 10:1055–1063.
- Gamblin TC, King ME, Dawson H, Vitek MP, Kuret J, Berry RW, Binder LI. In vitro polymerization of tau protein monitored by laser light scattering: method and application to the study of FTDP-17 mutants. *Biochemistry (Mosc)*. 2000; 39:6136–6144.
- Garcia-Sierra F, Ghoshal N, Quinn B, Berry RW, Binder LI. Conformational changes and truncation of tau protein during tangle evolution in Alzheimer's disease. *J Alzheimers Dis*. 2003; 5:65–77.

- Geddes JF, Vowles GH, Nicoll JA, Revesz T. Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol(Berl)*. 1999; 98:171–178. [PubMed: 10442557]
- Geddes JF, Vowles GH, Robinson SF, Sutcliffe JC. Neurofibrillary tangles, but not Alzheimer-type pathology, in a young boxer. *NeuropatholApplNeurobiol*. 1996; 22:12–16.
- Goedert M, Hasegawa M. The tauopathies: toward an experimental animal model. *AmJPathol*. 1999; 154:1–6.
- Gomez-Ramos P, Asuncion Moran M. Ultrastructural localization of intraneuronal Abeta-peptide in Alzheimer disease brains. *J Alzheimers Dis*. 2007; 11:53–59. [PubMed: 17361035]
- Gordon MN, King DL, Diamond DM, Jantzen PT, Boyett KV, Hope CE, Hatcher JM, DiCarlo G, Gottschall WP, Morgan D, Arendash GW. Correlation between cognitive deficits and Abeta deposits in transgenic APP+PS1 mice. *NeurobiolAging*. 2001; 22:377–385.
- Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science*. 1998; 280:1610–1613. [PubMed: 9616125]
- Guo Z, Cupples LA, Kurz A, Auerbach SH, Volicer L, Chui H, Green RC, Sadovnick AD, Duara R, DeCarli C, Johnson K, Go RC, Growdon JH, Haines JL, Kukull WA, Farrer LA. Head injury and the risk of AD in the MIRAGE study. *Neurology*. 2000; 54:1316–1323. [PubMed: 10746604]
- Hamm RJ, Pike BR, O'Dell DM, Lyeth BG, Jenkins LW. The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. *JNeurotrauma*. 1994; 11:187–196. [PubMed: 7932797]
- Hasegawa M, Smith MJ, Goedert M. Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. *FEBS Lett*. 1998; 437:207–210. [PubMed: 9824291]
- Hernandez F, Avila J. Tauopathies. *Cell Mol Life Sci*. 2007; 64:2219–2233. [PubMed: 17604998]
- Heyman A, Wilkinson WE, Stafford JA, Helms MJ, Sigmon AH, Weinberg T. Alzheimer's disease: a study of epidemiological aspects. *AnnNeurol*. 1984; 15:335–341.
- Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet*. 2008; 372:216–223. [PubMed: 18640458]
- Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, Mackey B, Olney J, McKeel D, Wozniak D, Paul SM. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2000; 97:2892–2897. [PubMed: 10694577]
- Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, Morris JC, Wilhelmsen KC, Schellenberg GD, Trojanowski JQ, Lee VM. Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science*. 1998; 282:1914–1917. [PubMed: 9836646]
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science*. 1996; 274:99–102. [PubMed: 8810256]
- Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol*. 1997; 56:965–973. [PubMed: 9291938]
- Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*. 2000; 408:979–982. [PubMed: 11140685]
- Jones RS. Entorhinal-hippocampal connections: a speculative view of their function. *Trends Neurosci*. 1993; 16:58–64. [PubMed: 7680501]
- Jordan BD. Chronic traumatic brain injury associated with boxing. *SeminNeurol*. 2000; 20:179–185.
- Katsuse O, Iseki E, Arai T, Akiyama H, Togo T, Uchikado H, Kato M, de Silva R, Lees A, Kosaka K. 4-repeat tauopathy sharing pathological and biochemical features of corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol*. 2003; 106:251–260. [PubMed: 12802605]

- Kempf M, Clement A, Faissner A, Lee G, Brandt R. Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. *J Neurosci*. 1996; 16:5583–5592. [PubMed: 8795614]
- Kobayashi DT, Chen KS. Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes Brain Behav*. 2005; 4:173–196. [PubMed: 15810905]
- Kosaka S, Takuma H, Tomiyama T, Mori H. The distributions of tau short and long isoforms fused with EGFP in cultured cells. *Osaka City Med J*. 2004; 50:19–27. [PubMed: 15646255]
- LaFerla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci*. 2007; 8:499–509. [PubMed: 17551515]
- Lynch JR, Pineda JA, Morgan D, Zhang L, Warner DS, Benveniste H, Laskowitz DT. Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Ann Neurol*. 2002; 51:113–117.
- Lynch JR, Wang H, Mace B, Leinenweber S, Warner DS, Bennett ER, Vitek MP, McKenna S, Laskowitz DT. A novel therapeutic derived from apolipoprotein E reduces brain inflammation and improves outcome after closed head injury. *Exp Neurol*. 2005; 192:109–116. [PubMed: 15698624]
- Mandelkow EM, Stamer K, Vogel R, Thies E, Mandelkow E. Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiol Aging*. 2003; 24:1079–1085. [PubMed: 14643379]
- Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg*. 1994; 80:291–300. [PubMed: 8283269]
- Masliah E, Mallory M, Deerinck T, DeTeresa R, Lamont S, Miller A, Terry RD, Carragher B, Ellisman M. Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. *J Neuropathol Exp Neurol*. 1993; 52:619–632. [PubMed: 8229081]
- Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein and Alzheimer's disease. *J Neurosci*. 1996; 16:5795–5811. [PubMed: 8795633]
- Miao J, Vitek MP, Xu F, Previti ML, Davis J, Van Nostrand WE. Reducing cerebral microvascular amyloid-beta protein deposition diminishes regional neuroinflammation in vasculotropic mutant amyloid precursor protein transgenic mice. *J Neurosci*. 2005; 25:6271–6277. [PubMed: 16000616]
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature*. 2000; 408:982–985. [PubMed: 11140686]
- Morris HR, Osaki Y, Holton J, Lees AJ, Wood NW, Revesz T, Quinn N. Tau exon 10 +16 mutation FTDP-17 presenting clinically as sporadic young onset PSP. *Neurology*. 2003; 61:102–104. [PubMed: 12847166]
- Mortimer JA, French LR, Hutton JT, Schuman LM. Head injury as a risk factor for Alzheimer's disease. *Neurology*. 1985; 35:264–267. [PubMed: 3969219]
- Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol*. 2009; 66:435–440. [PubMed: 19204149]
- Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, Phillips C, Gau BA, Welsh-Bohmer KA, Burke JR, Guralnik JM, Breitner JC. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology*. 2000; 55:1158–1166. [PubMed: 11071494]
- Probst A, Gotz J, Wiederhold KH, Tolnay M, Mistl C, Jaton AL, Hong M, Ishihara T, Lee VM, Trojanowski JQ, Jakes R, Crowther RA, Spillantini MG, Burki K, Goedert M. Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta Neuropathol*. 2000; 99:469–481. [PubMed: 10805089]
- Qing H, He G, Ly PT, Fox CJ, Staufenbiel M, Cai F, Zhang Z, Wei S, Sun X, Chen CH, Zhou W, Wang K, Song W. Valproic acid inhibits Abeta production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models. *J Exp Med*. 2008; 205:2781–2789. [PubMed: 18955571]

- Rademakers R, Cruts M, van Broeckhoven C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. *Hum Mutat.* 2004; 24:277–295. [PubMed: 15365985]
- Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. *Science.* 2002; 296:868–871. [PubMed: 11988563]
- Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to beta -amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A.* 2002; 99:6364–6369. [PubMed: 11959919]
- Reed LA, Wszolek ZK, Hutton M. Phenotypic correlations in FTDP-17. *Neurobiol Aging.* 2001; 22:89–107. [PubMed: 11164280]
- Roberson ED, Scarce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science.* 2007; 316:750–754. [PubMed: 17478722]
- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandever C, Walker S, Wogulis M, Yednock T, Games D, Seubert P. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature.* 1999; 400:173–177. [PubMed: 10408445]
- Schmued LC, Hopkins KJ. Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res.* 2000; 874:123–130. [PubMed: 10960596]
- Sergeant N, Wattez A, Delacourte A. Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively “exon 10” isoforms. *J Neurochem.* 1999; 72:1243–1249. [PubMed: 10037497]
- Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J Cell Biol.* 2002; 156:1051–1063. [PubMed: 11901170]
- Stokin GB, Almenar-Queralt A, Gunawardena S, Rodrigues EM, Falzone T, Kim J, Lillo C, Mount SL, Roberts EA, McGowan E, Williams DS, Goldstein LS. Amyloid precursor protein-induced axonopathies are independent of amyloid-beta peptides. *Hum Mol Genet.* 2008; 17:3474–3486. [PubMed: 18694898]
- Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science.* 2005; 307:1282–1288. [PubMed: 15731448]
- Takahashi T, Yagishita S, Amano N, Yamaoka K, Kamei T. Amyotrophic lateral sclerosis with numerous axonal spheroids in the corticospinal tract and massive degeneration of the cortex. *Acta Neuropathol.* 1997; 94:294–299. [PubMed: 9292701]
- Terry RD. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. *J Neuropathol Exp Neurol.* 1996; 55:1023–1025. [PubMed: 8857998]
- Togo T, Dickson DW. Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process. *Acta Neuropathol(Berl).* 2002; 104:398–402. [PubMed: 12200627]
- Vershinin M, Carter BC, Razafsky DS, King SJ, Gross SP. Multiple-motor based transport and its regulation by Tau. *Proc Natl Acad Sci U S A.* 2007; 104:87–92. [PubMed: 17190808]
- Vershinin M, Xu J, Razafsky DS, King SJ, Gross SP. Tuning microtubule-based transport through filamentous MAPs: the problem of dynein. *Traffic.* 2008; 9:882–892. [PubMed: 18373727]
- Villain N, Desgranges B, Viader F, de la Sayette V, Mezenge F, Landeau B, Baron JC, Eustache F, Chetelat G. Relationships between hippocampal atrophy, white matter disruption, and gray matter hypometabolism in Alzheimer's disease. *J Neurosci.* 2008; 28:6174–6181. [PubMed: 18550759]
- Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci U S A.* 1975; 72:1858–1862. [PubMed: 1057175]
- Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D. Passive immunotherapy against Aβ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation.* 2004; 1:24. [PubMed: 1558287]

- Wirths O, Breyhan H, Schafer S, Roth C, Bayer TA. Deficits in working memory and motor performance in the APP/PS1ki mouse model for Alzheimer's disease. *Neurobiol Aging*. 2008; 29:891–901. [PubMed: 17215062]
- Wirths O, Multhaup G, Bayer TA. A modified beta-amyloid hypothesis: intraneuronal accumulation of the beta-amyloid peptide--the first step of a fatal cascade. *J Neurochem*. 2004; 91:513–520. [PubMed: 15485483]
- Wirths O, Weis J, Szczygielski J, Multhaup G, Bayer TA. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol*. 2006; 111:312–319. [PubMed: 16520967]
- Wozniak DF, Brosnan-Watters G, Nardi A, McEwen M, Corso TD, Olney JW, Fix AS. MK-801 neurotoxicity in male mice: histologic effects and chronic impairment in spatial learning. *Brain Res*. 1996; 707:165–179. [PubMed: 8919293]
- Zhukareva V, Vogelsberg-Ragaglia V, Van Deerlin VM, Bruce J, Shuck T, Grossman M, Clark CM, Arnold SE, Masliah E, Galasko D, Trojanowski JQ, Lee VM. Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. *Ann Neurol*. 2001; 49:165–175. [PubMed: 11220736]

Figure 1. Profound neuritic dystrophy in $A_{sw}/mTau^{-/-}$ mice

de Olmos silver stain of 12 month old mice revealed extensive neuritic dystrophy (in the form of spheroids) in the $A_{sw}/mTau^{-/-}$ mouse brains (C., F., I.). Silver positive amyloid plaques are visible in image D. and F (arrowheads). The A_{sw} (A, D, G) or $mTau^{-/-}$ (B, E, H) mouse control brains show minimal silver positive spheroids. CC: corpus callosum, S: subiculum, IC: internal capsule labeled for reference.

Figure 2. de Olmos stained images of spheroids and plaques in $A_{SW}/mTau^{-/-}$ mice
 A. . 10 $\times$ image of the genu of the corpus callosum (lower right corner) depicting spheroids (radiating from the corpus callosum into the surrounding cortex) and two large plaques (upper left side). B. 60x image of spheroids (open arrows), degenerating axons (arrows) and small axonal swellings (arrow heads). C. Enlarged image of a neuritic plaque from A. (asterisk) depicting an associated neurite with multiple swellings/spheroids (arrowhead). D. Image of a non-neuritic plaque. It is important to note the varying sizes of spheroids indicative of axonal swellings.

Figure 3. The amount of spheroids is age dependent in the A_{SW}/mTau^{-/-} mice

The number of spheroids surrounding the genu of the corpus callosum was counted in 6 brain slices per animal using unbiased stereology. The A_{SW}/mTau^{-/-} brains show a clear increase of spheroids at 12 month of age while the A_{SW} and A_{SW}/Tau^{-/-}/hTau^{+/-} mice show a reduction of spheroids. No change was detected in Tau^{-/-} mice. * p<0. 05, ** p<0. 01, *** p<0. 001.

Figure 4. Ultrastructural analysis of degenerating axons in $A_{sw}/mTau^{-/-}$ mice
 A. Transmission electron microscopy revealed a spheroid (center of image) filled with degenerating mitochondria, vesicular and membranous multilamellar structures and vacuoles. Normal myelinated transverse axons (*) encased in myelin (arrowheads) and containing normal mitochondria (arrows) surround this spheroid. B. An unmyelinated axonal spheroid (center of image), filled with condensed fibrils and filaments, is surrounded by cross sectioned normal unmyelinated axons (arrows). The asterisk (*) depicts a normal axonal terminal. A. 7,100X and B. 12,000X.

Figure 5. Fluoro-jade B stained images from A_{sw}/mTau^{-/-} mice

A. 20x image of the genu of the corpus callosum (CC) and surrounding striatum (S) and cortex (C). Arrows point to spheroids, arrowhead points to spheroids in a striatal bundle. B. 60x image of spheroids in the cortex (arrows point to some of the spheroids with associated neurites). C. 60x image of a striatal bundle with spheroids (arrows). D. 60x image of a neuritic plaque (■) surrounded by spheroids (arrows) and 2 astrocytes (*). It is important to note the varying sizes of spheroids in all images indicative of axonal swellings.

Figure 6. A subset of spheroids is axonal in origin

A. Spheroids (arrows) in the brains of $A_{SW}/mTau^{-/-}$ mice were immunopositive with a synaptophysin antibody surrounding the genu of the corpus callosum. Arrowheads in A indicate non-specific staining of blood vessels. B. Synaptophysin immunopositive spheroids (arrows) in the anterior thalamus, inset shows a spheroid at higher magnification indicated by the double arrow. Cortex (C), corpus callosum(CC), striatum (S), interior capsule (IC), and anterior thalamus (AN).

Figure 7. The loss of tau decreases the neuritic plaque load but increases A β

Using unbiased stereology, the plaque area was measured in the cortex and hippocampus and is represented as a fraction of the total cortex and hippocampal area. A. There is a significant decrease in the combined areas of cortex and hippocampus of the area that contains neuritic plaques in the brains of A_{sw}/mTau^{-/-} mice compared to A_{sw} mice at both 10 and 12 months. B. No statistically significant increase between A_{sw} and A_{sw}/mTau^{-/-} mice is seen in non-neuritic plaques. The area of both neuritic plaques A., and non-neuritic plaques B., increases with age in both the A_{sw} and A_{sw}/mTau^{-/-} mice although the increase is not always statistically significant, * 0.01 ≤ p ≤ 0.05. C. Western blot analysis showing the expression of tau protein and APP protein in; Lane 1 WT, Lane 2 A_{sw}, Lane 3 mTau^{-/-}, and Lane 4 A_{sw}/mTau^{-/-}/hTau^{+/+} mice. TAU17026 is a pan tau antibody, TAU14 is a human specific antibody and APP is a pan APP antibody. GAPDH was used to ensure equal loading. D. Quantitative results of soluble (s) and insoluble (i) beta amyloid peptides 40 and 42 in whole brains of A_{sw} and A_{sw}/mTau^{-/-} mice (***p < 0.0001). E. Western blot analysis of A_{sw} (lanes 1–3) and A_{sw}/mTau^{-/-} (lanes 4–7) mice shows no difference in the expression of APP with the 6E10 N-terminal or APP-C C-terminal antibodies. There is no detectable expression of APP in WT (lanes 8–10) or mTau^{-/-} (lanes 11–13) mice at this level of exposure. F. Semi-quantification of the expression of APP depicted in E. normalized to GFAP and shown as a percentage of A_{sw} mouse APP expression (p = 0.29 and p = 0.41 for 6E10 and APP-C respectively).

Figure 8. APP and/or A β accumulate in neurons

A. – C. Images of A_{sw}/mTau^{-/-} mouse brain. A. 4G8 immunopositive accumulation is evident in the neurons of the subiculum and CA1 pyramidal layer of the hippocampus, arrow indicates the subiculum/CA1 junction, CC indicates the corpus callosum (20x image). B. A 60x magnification of the subiculum/CA1 junction shown (arrow) in A. Note the punctate nature of the intracellular 4G8 immunostaining. C. 4G8 immunopositive accumulation is also present in layer 5 of the cortex (60x image). D. No immunopositive 4G8 staining is evident in the CA1 region of the hippocampus or in the cortex (C) of A_{sw} mice except for plaques (arrowhead), CC, corpus callosum.

Figure 9. $A_{sw}/mTau^{-/-}$ mice have vestibulomotor and cognitive deficits
The table in panel D displays the number of animals used in each behavioral experiment. A. The $A_{sw}/mTau^{-/-}$ mice are deficient in the Morris water maze when compared to the other 3 experimental groups, p value was $p < 0.0001$ for all the groups. B. Radial arm water maze shows that both the $A_{sw}/mTau^{-/-}$ and A_{sw} mice have cognitive deficits with respect to $mTau^{-/-}$ or WT mice, ANOVA $p < 0.05$. However, while the WT, $mTau^{-/-}$ and the A_{sw} mice all improve in the task between day 1 and the probe trial ($p < 0.007$), the $A_{sw}/mTau^{-/-}$ mice do not, t-test $p = 0.207$. C. The $A_{sw}/mTau^{-/-}$ mice are deficient on the rotarod when compared to their controls, t-test.

SPHEROIDS - HEAD INJURY

Figure 10. Head injury increases axonal degeneration in $A_{sw}/mTau^{-/-}$ mice
 A_{sw} , $mTau^{-/-}$, $A_{sw}/Tau^{-/-}$ and $A_{sw}/Tau^{-/-}/hTau^{+/-}$ mice were challenged with traumatic brain injury, a predisposing factor for some tauopathies. At 10 month of age the number of spheroids (per brain slice) increase dramatically in the $A_{sw}/mTau^{-/-}$ mice but remained unchanged in the single transgenic controls, this increase is prevented with the reconstitution of tau function, $A_{sw}/mTau^{-/-}/hTau^{+/-}$ mice.

Figure 11. Immunization with Aβ does not improve outcomes in A_{sw}/mTau^{-/-} mice

A. Immunization with Aβ reduces both the neuritic and non-neuritic plaque load in A_{sw} mice ($p=0.014$ and $p=0.035$ respectively) only the non-neuritic but not neuritic plaque load in A_{sw}/mTau^{-/-} mice is decreased ($p=0.0244$ and $p=0.6048$ respectively). B. The number of spheroids remains unchanged in A_{sw}/mTau^{-/-} mice after Aβ immunization ($p=0.1658$). C. Aβ immunization of A_{sw} mice improves their ability to learn the radial arm water maze (ANOVA, $p=0.0051$) while it decreases D. the ability of the A_{sw}/mTau^{-/-} mice to perform on the radial arm water maze (ANOVA, $p=0.0337$). E. Quantitative results of soluble (s) and insoluble (i) Aβ 40 and 42 in whole brains of A_{sw} and A_{sw}/mTau^{-/-} mice. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 1

Unbiased stereological quantification of spheroids.

	A _{sw}	n	mTau ^{-/-}	n	A _{sw} /mTau ^{-/-}	n	A _{sw} /mTau ^{-/-} /hTau ^{+/-}	n
10 MONTHS	35 ± 4	4	66 ± 9	5	56 ± 7	5	29 ± 7	3
12 MONTHS	9 ± 1	3	63 ± 11	4	314 ± 24	6	11 ± 1	3
HEAD INJURY	50 ± 12	4	50 ± 5	5	214 ± 34	4	14 ± 14	3
Aβ Immunization	14 ± 1	7	na [*]		265 ± 22	9	na [*]	

Numbers are presented as average number of Spheroids per slice, n= the number of mice per experiment

^{*} Experiment not performed

Unbiased stereology quantification of the average fraction of cortical and hippocampal area containing plaques

Table 2

	NEURITIC PLAQUES			NON-NEURITIC PLAQUES		
	A _{sw}	n	A _{sw} /mTau ^{+/+}	A _{sw}	n	A _{sw} /mTau ^{+/+}
10 MONTHS	6.8 ± 2.3	5	0.77 ± 0.069	1.7 ± 1.2	5	4.2 ± 1.5
12 MONTHS	19 ± 8.4	6	4.4 ± 0.94	47 ± 22	6	29 ± 9.7
Aβ Immunization	3.5 ± 3.0	6	4.9 ± 2.10	1.5 ± 1.2	6	16 ± 1.40
HEAD INJURY	1.3 ± 0.8	4	2.3 ± 0.92	0.01 ± 0.01	4	0.08 ± 0.03

Plaques are represented by percentage of total area covered by plaques; all numbers are expressed as 10⁻⁴
n= the number of mice per experiment

Table 3ELISA quantification of A β levels in the brains of APP overexpressing mice (pg/mg)

A β Fraction	A _{sw}	A _{sw} + A β	A _{sw} /mTau ^{-/-}	A _{sw} /mTau ^{-/-} + A β
42s	67 $\pm$ 9	9 $\pm$ 2	181 $\pm$ 9	17 $\pm$ 2
40s	150 $\pm$ 22	30 $\pm$ 11	506 $\pm$ 8	95 $\pm$ 24
42i	210 $\pm$ 44	22 $\pm$ 11	1049 $\pm$ 94	485 $\pm$ 327
40i	235 $\pm$ 37	95 $\pm$ 39	2834 $\pm$ 78	857 $\pm$ 308
42s+42i	277 $\pm$ 52	30 $\pm$ 10	1230 $\pm$ 103	502 $\pm$ 327
40s+40i	385 $\pm$ 54	125 $\pm$ 36	3340 $\pm$ 84	952 $\pm$ 330
TOTAL Aβ	662 $\pm$ 105	156 $\pm$ 46	4571 $\pm$ 184	1455 $\pm$ 651

Values are given as pg of A β plus or minus standard error.40 = A β ₁₋₄₀, 42=A β ₁₋₄₂, s=soluble fraction, i=insoluble fraction