Animal Models

Lack of Tau Proteins Rescues Neuronal Cell Death and Decreases Amyloidogenic Processing of APP in APP/PS1 Mice

Karelle Leroy,* Kunie Ando,* Vincent Laporte,[†]
Robert Dedecker,* Valérie Suain,*
Michèle Authélet,* Céline Héraud,*
Nathalie Pierrot,[†] Zehra Yilmaz,*
Jean-Noël Octave,[†] and Jean-Pierre Brion*

From the Laboratory of Histology, Neuroanatomy, and
Neuropathology,* Université Libre de Bruxelles, Brussels; and the
Institute of Neuroscience,[†] Université Catholique de Louvain,
Brussels, Belgium

Lack of tau expression has been reported to protect against excitotoxicity and to prevent memory deficits in mice expressing mutant amyloid precursor protein (APP) identified in familial Alzheimer disease. In APP mice, mutant presenilin 1 (PS1) enhances generation of A β 42 and inhibits cell survival pathways. It is unknown whether the deficient phenotype induced by concomitant expression of mutant PS1 is rescued by absence of tau. In this study, we have analyzed the effect of tau deletion in mice expressing mutant APP and PS1. Although APP/PS1/tau^{+/+} mice had a reduced survival, developed spatial memory deficits at 6 months and motor impairments at 12 months, these deficits were rescued in APP/PS1/tau^{-/-} mice. Neuronal loss and synaptic loss in APP/PS1/tau^{+/+} mice were rescued in the APP/PS1/tau^{-/-} mice. The amyloid plaque burden was decreased by roughly 50% in the cortex and the spinal cord of the APP/PS1/tau^{-/-} mice. The levels of soluble and insoluble A β 40 and A β 42, and the A β 42/A β 40 ratio were reduced in APP/PS1/tau^{-/-} mice. Levels of phosphorylated APP, of β -C-terminal fragments (CTFs), and of β -secretase 1 (BACE1) were also reduced, suggesting that β -secretase cleavage of APP was reduced in APP/PS1/tau^{-/-} mice. Our results indicate that tau deletion had a protective effect against amyloid induced toxicity even in the presence of mutant PS1 and reduced the production of A β . (Am J Pathol 2012, 181:1928–1940; <http://dx.doi.org/10.1016/j.ajpath.2012.08.012>)

Alzheimer disease (AD) is neuropathologically characterized by the presence of neurofibrillary tangles and senile plaques. Neurofibrillary tangles consist of paired helical filaments, composed of abnormally phosphorylated forms of the microtubule-associated protein tau. Senile plaques have an extracellular core of amyloid surrounded by dystrophic neurites and reactive glial cells. The amyloid core is mainly composed of fibrillar aggregates of the amyloid- β peptide (A β), generated by proteolytic cleavage of the transmembrane amyloid precursor protein (APP). APP is thought to play a central role in the disease, eg, in view of familial forms of AD due to mutations in APP. According to the amyloid cascade hypothesis, the A β peptide, or oligomeric forms of it, is neurotoxic, leading to synaptic dysfunction, neuronal death, and formation of neurofibrillary tangles. Several results, however, have suggested that tau might influence the development of behavioral deficits and cellular abnormalities associated with amyloid pathology. For instance, cultured neurons treated with fibrillar A β showed signs of degeneration, whereas neurons with a knockout tau gene were resistant to A β toxicity.¹ Also, tau was found to confer to microtubules hypersensitivity to depolymerization induced by prefibrillar A β in cultured cells.² Tau reduction in transgenic mice overexpressing mutant APP prevented the development of learning and memory deficits and increased their resistance to excitotoxin-induced seizures.³ Absence of tau has been suggested to reduce A β toxicity at the postsynaptic level by uncoupling NMDA receptor-mediated excitotoxicity.⁴ A β impairs long-term potentiation,⁵ and tau has been reported to be required for this A β -induced impairment of long-

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Address reprint requests to Jean-Pierre Brion, M.D., Ph.D., Laboratory of Histology, Neuroanatomy, and Neuropathology, School of Medicine, Université Libre de Bruxelles, 808, route de Lennik, Bldg GE, 1070 Brussels, Belgium. E-mail: jbrion@ulb.ac.be.

term potentiation,⁶ most probably through GSK-3 β .^{6,7} Tau reduction also prevented A β -induced defects in axonal transport⁸ and behavioral deficits in APPxFyn mice.⁹ Deletion of *tau* in another APP mouse model led to an increased axonal degeneration instead.¹⁰ Presenilin 1 (PS1) is necessary for γ -cleavage of APP and generation of A β , and mutant PS1 (found in familial forms of AD) enhances formation of A β 42 in mice overexpressing mutant APP. PS1, however, also binds both tau and GSK-3 β , and mutant PS1 binds more GSK-3 β , inducing increased tau phosphorylation.¹¹ Wild-type PS1 prevents apoptosis by activating PI3K/Akt signaling and inhibiting GSK-3 β activity, whereas the mutant form of PS1 inhibits PI3K/Akt cell survival pathway by promoting GSK-3 β activity.¹² Thus, mutant PS1 affects amyloidogenic processing of APP but has additional deleterious effects on cell survival.

To further analyze the link between tau expression and APP/A β toxicity, neuronal loss, and APP processing, and to know whether the protective effect of tau reduction was also present when mutant forms of PS1 were expressed, we have generated a new model expressing both of the mutants APP and PS1 in absence of tau. We observed that tau inactivation, not only improved memory and motor deficits in APP/PS1 mice, but also rescued neuronal cell death and decreased A β load.

Materials and Methods

Generation of Transgenic Mice

The 5xFAD (hereafter called APP/PS1/*tau*^{+/+}) heterozygote Tg6799 mice contain five familial AD mutations for APP (K670N/M671L, I716V, and V717I) and for PS1 (M146L and L286V).¹³ Mutant APP and PS1 transgene expression is driven by the mouse *Thy1* promoter. The *tau*^{-/-} mouse line (the Jackson Laboratory, Bar Harbor, ME) was generated by knockin of the EGFP coding sequence into the first exon of the tau gene.¹⁴ Both the APP/PS1 and *tau*^{-/-} lines were maintained on a C57BL6 background. APP/PS1/*tau*^{+/+} mice were crossed with *tau*^{-/-} mice to generate F₁ APP/PS1/*tau*^{+/-} mice. APP/PS1/*tau*^{+/-} mice were then bred with *tau*^{+/-} mice to generate F₂ APP/PS1/*tau*^{-/-} mice and littermates APP/PS1/*tau*^{+/+}, APP/PS1/*tau*^{+/-}, *tau*^{-/-}, *tau*^{+/-}, and *tau*^{+/+} (wild-type) mice with the same genetic background. Only the representative four genotypes (*tau*^{+/+}, *tau*^{-/-}, APP/PS1/*tau*^{+/+}, and APP/PS1/*tau*^{-/-}) from the same littermates were further analyzed. Genotyping was performed by three independent PCR amplifications of DNA extracted from tail DNA using previously described primers for human APP,¹³ murine *tau*, and GFP.¹⁵ All studies on animals were performed in compliance and following approval of the ethical committee for the care and use of laboratory animals of the Medical School of the Free University of Brussels.

Motor and Behavioral Testing

Motor deficits were assessed with the rotarod test, as previously described.^{15,16} Mice were placed on a rod

rotating with a progressive acceleration from 4 to 40 rpm in 5 minutes. Mice were first trained with three trials per day during 3 days, and the latency that mice spent on the rotating rod before falling was measured on the third day. Spatial working memory was investigated as previously described using a Y maze and assessment of spontaneous alternation.¹⁷ Each mouse was placed at the end of one arm and was allowed to explore freely for 8 minutes, during which their movements were recorded with a digital camera. Records were analyzed by an observer blinded to the genotype that encoded the sequence and the total number of arms entered offline. An entry was considered when the four paws were in the arm. Spontaneous alternation was computed as the number of triads containing entries in the three arms divided by the total number of arms minus two.

Antibodies

The B19 antibody is a rabbit polyclonal antibody raised to adult bovine tau proteins, reacting with adult and fetal tau isoforms in bovine, rat, mouse, and human nervous tissue in a phosphorylation-independent manner.¹⁸ The mouse monoclonal anti-APP antibody 3H5 is specific for human APP.¹⁹ The BR15 rabbit polyclonal antibody was raised against a synthetic peptide corresponding to the last 20 amino acids of human APP, conjugated to purified protein derivative, and reacts with mouse and human APP. The rabbit polyclonal APP antibodies APPCter-C17 (to the C-terminus of human APP) and APP668P (to a human APP peptide phosphorylated on its Thr668) were kindly provided by Drs. Nicolas Sergeant and Luc Buée (INSERM U837, France).²⁰ For detection of A β , we used a rabbit polyclonal antibody specific for A β 40 (BioSource; purchased from Invitrogen, Carlsbad, CA), a rabbit polyclonal antibody specific for A β 42 (purchased from Millipore, Billerica, MA), and a mouse monoclonal antibody raised to amino acids 1 to 16 of the A β peptide (6E10; purchased from Covance, Princeton, NJ). A rat monoclonal antibody to human PS1 was purchased from Chemicon (MAB1563; Chemicon/Millipore, Billerica, MA). The rabbit polyclonal anti-PS1 antibody (SB129) recognizes both human and murine PS1 (generously provided by Dr. Christine Van Broeckhoven, UIA, Belgium).²¹ The BR6 rabbit polyclonal antibody was raised against cathepsin B from bovine spleen (Sigma C6286; Sigma-Aldrich, St. Louis, MO), and reacts with mouse and human cathepsin B. The following additional antibodies were also used: mouse monoclonal antibodies to α -tubulin (clone DM1-A) and to β -actin (clone AC15) (Sigma-Aldrich, Bornem, Belgium), to GSK-3 β (TPK1) (BD Transduction Laboratories; BD Biosciences, San Jose, CA), to synaptophysin (clone YE269) and PSD95 (clone K28/43) (Millipore), rabbit polyclonal antibodies to BACE1 (Rockland Immunochemicals, Gilbertsville, PA), to GSK-3 α/β phosphorylated on Ser21/9 (Cell Signaling Technology, Danvers, MA), and to GSK-3 α/β phosphorylated on Tyr276/Tyr216 (BioSource; Invitrogen).

Preparation of Brain and Spinal Cord Homogenates

Samples were homogenized in 10 volumes of radioimmuno-precipitation assay (RIPA) buffer [50 mmol/L Tris (pH 7.4) containing 150 mmol/L NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 25 μ g/mL leupeptin, 25 μ g/mL pepstatin, 10 mmol/L sodium pyrophosphate, 20 mmol/L sodium fluoride, 1 mmol/L sodium orthovanadate] for brains and in 20 volumes for the spinal cords. The homogenate was centrifuged ($20,000 \times g$ for 20 minutes at 4°C), and the supernatant was used as a soluble fraction. The pellet was resuspended in threefold volume of 100% formic acid by vigorous pipetting and vortexing, followed by incubation overnight at 4°C on a rotator. The mixture was centrifuged at $20,000 \times g$ at 4°C for 20 minutes. The supernatant was neutralized with 19 volumes of 2 mol/L Tris buffer. This formic acid fraction was used as the insoluble fraction.

Western Blot Analysis

Protein concentrations were estimated by the Bradford method (Bio-Rad Laboratories, Hercules, CA). Tissue samples (100 μ g/lane) were run in either 7.5% or 10% Tris-glycine gels or 16% Tris-Tricine gels and then transferred onto nitrocellulose membranes. The nitrocellulose sheets were blocked in semifat dry milk [10% (w/v) in Tris-buffered saline] for 1 hour at room temperature and then incubated with primary antibodies overnight followed by anti-rabbit or anti-mouse immunoglobulins conjugated to alkaline phosphatase (Sigma-Aldrich) or peroxidase (for the electrochemiluminescence method). Finally, the membranes were incubated in developing buffer [0.1 mol/L Tris, 0.1 mol/L NaCl, 0.05 mol/L $MgCl_2$ (pH 9.5)] containing nitro blue tetrazolium at a concentration of 0.33 mg/mL and 5-bromo-4-chloro-3-indolyl phosphate at a concentration of 0.175 mg/mL or in SuperSignal West Pico substrate (Pierce Biotechnology, Thermo Fisher Scientific, Waltham, MA). The development was stopped by dipping the membranes in 10 mmol/L Tris, 1 mmol/L EDTA (pH 8) or after exposure of an X-ray film. The levels of expression of transgenic proteins and fragments of APP were estimated by densitometry analysis using the NIH Image J program (NIH, Bethesda, MD), and adjusted for protein loading based on immunoblots performed with the anti-actin antibody.

Immunoprecipitation

Co-immunoprecipitations for PS1, APP, and GSK-3 β were performed using a Rabbit TrueBlot IP kit (eBioscience, San Diego, CA) following the manufacturer's instructions. Briefly, the RIPA soluble fraction was pre-cleared with pre-equilibrated Rabbit TrueBlot bead slurry. Pre-cleared samples were incubated overnight on a rotator with the anti-PS1 SB129 antibody. Protein complexes were precipitated with Rabbit TrueBlot beads followed by repetitive washes. After the third wash, SDS sample buffer containing 5% β -mercaptoethanol was

added to the beads, and the samples were heated at 37°C for PS1 and at 100°C for the other proteins.

ELISA

In the soluble and insoluble fractions of brain and spinal cord of 6-month-old mice, we measured the concentration of three species of β -amyloid peptide, A β 38, A β 40, and A β 42, by electrochemiluminescence immunoassay. Its principle was described by Best et al,²² and the assays were performed according to the manufacturer's instructions. Briefly, samples were analyzed using Meso Scale Discovery SECTOR Imager 2400 [Meso Scale Discovery (MSD), Gaithersburg, MD], with the human A β triplex kit (also from MSD); the 96-well carbon electrode plates contained four capture spots in each well, one of which was blocked with bovine serum albumin (as the standard curve control), and the three others coated with isoform-specific anti-A β antibodies specific for A β 38, A β 40, A β 42, respectively. A total of 100 μ L of blocking buffer solution was added to all wells to avoid nonspecific binding. The plates were then sealed, wrapped in a thin foil, and then incubated at room temperature on a plate shaker (600 rpm) for 1 hour. At the end of the incubation, the wells were washed three times with washing buffer. The samples and 25 μ L of the standards (A β 38, A β 40, and A β 42) were then added to the wells, followed by an A β -detecting antibody (MSD) at 1 μ g/mL labeled with a ruthenium (II) tris-bipyridine *N*-hydroxysuccinimide ester; this detection antibody is 6E10 (recognizing, specifically, amino acids 3 to 8 of human A β). Plates were then aspirated and washed three times. MSD read buffer (containing tripropylamine) was added to the wells before reading on the Sector Imager, and a small electric current through the microelectrodes present in each well was used to produce a redox reaction of the Ru^{2+} cation, with emission of a measured 620-nm red light ray. Using dose-response curves, we calculated the concentration of each A β isoform for each sample, the blank being wild-type homogenates.

Histological Staining and Immunohistochemistry

Mice were sacrificed by cervical dislocation, and after dissection, the brains and spinal cords were fixed in 10% formalin for 24 hours before embedding in paraffin. Tissue sections (7 μ m thick) were stained with hematoxylin & eosin and the Nissl method for histological examination, and also with Congo Red and with thioflavin S for detection of aggregated amyloid deposits. For immunohistochemistry, sections were processed as previously described.²³ Briefly, they were incubated in H_2O_2 to inhibit endogenous peroxidase, rinsed in water, and then incubated with a blocking solution [10% of normal rabbit serum in TBS [0.01 mol/L Tris, 0.15 mol/L NaCl (pH 7.4)]]. The sections were then incubated overnight with the primary antibody and then incubated with anti-mouse or anti-rabbit antibodies conjugated to biotin (Vector Laboratories, Burlingame, CA) followed by the ABC complex (Vector Laboratories). Diaminobenzidine was used as a substrate for revealing peroxidase activity. Double immu-

nofluorescent labeling was performed as previously reported, using horse anti-mouse antibody conjugated to fluorescein isothiocyanate (the Jackson Laboratory) and horse anti-rabbit antibodies conjugated with biotin (Vector Laboratories) and streptavidin-conjugated Alexa594 (Molecular Probes/Invitrogen, Carlsbad, CA). For immunolabeling with the A β antibodies, rehydrated tissue sections were pre-treated with 100% formic acid for 10 minutes before incubation with the blocking solution. Tissue sections were examined with a Zeiss Axioplan microscope and digital images acquired using an Axiocam HRc camera, and an ApoTome system (Carl Zeiss, Oberkochen, Germany) for double immunofluorescent labeling.

Stereological Analysis

Formalin 10% (v/v) fixed and paraffin-embedded samples were serially cut in coronal sections (15 μ m thickness) and stained with Cresyl violet; stereological analysis was performed as reported previously.^{15,16} For the estimation of the neuron number in the pyramidal layer of the hippocampus in 12-month-old mice, we used 10 coronal sections at 225- μ m intervals for each animal ($n = 4$). The counting was performed with a 100 $\times$ oil objective with the Stereo Investigator software (version 9; MicroBrightField, Colchester, VT) on a motorized Zeiss Axio Imager M1 microscope. Optical disectors (30 μ m $\times$ 30 μ m $\times$ 9 μ m) were randomly and automatically distributed throughout the region of interest. Each pyramidal neuron whose nucleolus came into focus within an optical disector was counted. Estimation of the total number of pyramidal cells was calculated using the number of counted neurons and the volume of the pyramidal layer (Gundersen coefficient of error: 0.035 ± 0.009 ; Schmitz-Hof coefficient of error: 0.035 ± 0.007). An estimation of neuronal density was done on lumbar spinal cords using 11 cross sections at 75- μ m intervals for each animal ($n = 4$). Optical disector (50 μ m $\times$ 50 μ m $\times$ 12 μ m) were randomly and automatically distributed throughout the anterior horn of spinal cord. The density of neurons was calculated from the numbers of counted neurons per volume of spinal gray matter estimated according to Cavalieri's principle.

Quantification of Extracellular A β Plaque Load

Quantification of extracellular amyloid deposits after immunolabeling with anti-A β 40 and anti-A β 42 antibodies in 6-month-old mice was performed in the cortex on sagittal sections taken near the midline and in the cervical spinal cord on cross sections. Virtual slices of whole brain or spinal cord sections were acquired with a camera (CX9000) mounted onto a Zeiss Axio Imager M1 microscope using the Stereo Investigator program, and pictures were binarized to 8-bit black and white images with the Image J program (version 1.41; NIH). After delimiting the cortex, the hippocampus, and the gray matter of the spinal cord in each image, the A β immunolabeled areas were measured in these regions by detecting positive pixels using a constant threshold (manually optimized)

Figure 1. Levels of expression of APP and PS1 human transgenic proteins are similar in APP/PS1/*tau*^{+/+} and APP/PS1/*tau*^{-/-} mice. **A:** Western blots of brain homogenates of *tau*^{+/+}, *tau*^{-/-}, APP/PS1/*tau*^{+/+}, and APP/PS1/*tau*^{-/-} mice with antibodies against human APP (3H5), murine and human APP (BR15), tau (B19), murine and human PS1 (SB129), and human PS1 (MAB1563). An actin antibody was used as a control for protein load. Molecular weight markers: 148 kDa (phosphorylase), 98 kDa (bovine serum albumin), 64 kDa (glutamic dehydrogenase), 50 kDa (alcohol dehydrogenase), 36 kDa (carbonic anhydrase), and 22 kDa (myoglobin red). **B–E:** Densitometry analysis. The levels of human APP (**B**), total APP (murine and human) (**C**), human PS1 (holoprotein) (**D**), and human PS1 N-terminal fragment (**E**) are similar in APP/PS1/*tau*^{+/+} ($n = 7$) and APP/PS1/*tau*^{-/-} ($n = 7$) mice. Tau proteins are not expressed in *tau*^{-/-} and APP/PS1/*tau*^{-/-} mice.

with the Image J program, and were expressed as a percentage of the total surface of the cortex, the hippocampus, and the gray matter of the spinal cord. The density of amyloid plaques and their mean size were calculated from the number of objects selected by setting thresholds with the Image J program.

Figure 2. Neuronal expression of human APP and PS1 in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice. Immunocytochemical detection of human APP (3H5 antibody) (A, B, and C), PS1 (SB129 antibody) (D, E, and F), and tau (B19 antibody) (G, H, and I) in hippocampus of *tau*^{+/+} (A, D, and G), *APP/PS1/tau*^{+/+} (B, E, and H), and *APP/PS1/tau*^{-/-} (C, F, and I) mice (*n* = 3). Human APP and PS1 are expressed in neurons of the pyramidal layer in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice. The PS1 antibody also detects endogenous PS1 in *tau*^{+/+} mice (D). Tau immunoreactivity is strong in axonal Mossy fibers in *tau*^{+/+} and in *APP/PS1/tau*^{+/+} mice (G and H) but is absent in *APP/PS1/tau*^{-/-} mice (I). Scale bar = 50 μ m.

Analysis of Spine Density after Golgi Staining

Golgi-Cox impregnation and staining of 150 μ m thick tissue sections from brain and spinal cord were performed using the FD Rapid GolgiStain kit (FD NeuroTechnologies, Columbia, MD). Fully impregnated cortical neurons from layer 5 were randomly selected for spine density analysis using the Neurolucida program (version 9; MicroBrightField), using a 100 $\times$ oil objective. The first-order apical dendrite of each pyramidal neuron was selected and tracing started from its cell body origin to a distance of 150 μ m. Each dendritic spine along this dendritic segment was marked by an observer blinded to the genotype and spine density calculated for each neuron.

Ultrastructural Analysis in Electron Microscopy

Control and transgenic animals were anesthetized with ketamine hydrochloride and xylazine, and perfused intracardially with a solution of 2% (w/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.4. Tissue blocks of the spinal cord, hippocampus, and of the cerebral cortex were dissected and further fixed by immersion in 4% (w/v) glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.4 for 90 minutes. After washing in Millonig's buffer with 0.5% (w/v) sucrose for 24 hours, the tissue blocks were postfixed in 2% (w/v) OsO₄ for 30 minutes, dehydrated, and then embedded in Epon. Semithin sections were stained with toluidine blue. Ultrathin sections were counterstained with uranyl acetate and lead citrate, and observed with a Zeiss EM 809 transmission electron microscope at 80 kV.

Statistical Analysis

Statistical significance was determined by unpaired Student's *t*-test and by one-way or two-way analysis of vari-

ance (with Bonferroni post hoc test) using Prism 4 software (GraphPad Software, La Jolla, CA).

Results

Levels of Expression of APP and PS1 Are Similar in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} Mice

APP/PS1/tau^{-/-} mice bearing human APP and PS1 mutant transgenes and with two inactivated *tau* alleles were analyzed by comparison with their littermates. The expression profiles of APP, PS1, and tau proteins in brain homogenates of *tau*^{+/+} (wild-type), *tau*^{-/-}, *APP/PS1/tau*^{+/+}, and *APP/PS1/tau*^{-/-} mice are shown in Figure 1A. The expression levels of full-length APP detected by anti-human APP and anti-APP Cter antibodies and of PS1 (N-terminal fragment and holoprotein) were not different between *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice (Figure 1, B–E). Tau proteins were not expressed in *tau*^{-/-} and *APP/PS1/tau*^{-/-} mice. Similar expression profiles were obtained in the spinal cord (data not shown). Immunocytochemical labeling confirmed the neuronal expression of human APP (Figure 2, A–C) and PS1 (Figure 2, D–F) in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice, and the absence of expression of tau in *APP/PS1/tau*^{-/-} mice (Figure 2I). Dystrophic neurites around the plaques were labeled with the APP and PS1 antibodies in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} brains, and with tau antibodies in *APP/PS1/tau*^{+/+} brains.

Reduced Survival of *APP/PS1/tau*^{+/+} Mice Is Partially Rescued in the Absence of tau

Kaplan-Meier survival curves of *tau*^{+/+}, *tau*^{-/-}, *APP/PS1/tau*^{+/+}, and *APP/PS1/tau*^{-/-} mice were analyzed up to 12 months of age (Figure 3A). *APP/PS1/tau*^{+/+} mice, but not *APP/PS1/tau*^{-/-} mice, showed a significantly re-

Figure 3. Motor deficits, working memory deficits, and reduced survival in APP/PS1/tau^{+/+} mice are rescued in APP/PS1/tau^{-/-} mice. **A:** Kaplan-Meier survival curve of tau^{+/+} (n = 31), APP/PS1/tau^{+/+} (n = 25), tau^{-/-} (n = 21), and APP/PS1/tau^{-/-} (n = 28) mice. APP/PS1/tau^{+/+} mice, but not APP/PS1/tau^{-/-} mice, show a reduced survival compared to tau^{+/+} mice. **P < 0.01, by log-rank test. **B:** Rotarod testing of tau^{+/+} (n = 7), tau^{-/-} (n = 8), APP/PS1/tau^{+/+} (n = 12), and APP/PS1/tau^{-/-} (n = 12) mice at 12 months of age. The time spent on the rotating rod differs by genotype (APP/PS1 by tau interaction, P < 0.05, two-way analysis of variance); it is significantly reduced for the APP/PS1/tau^{+/+} mice compared to all other genotypes and is rescued in the APP/PS1/tau^{-/-} mice. *P < 0.05, Bonferroni post hoc test. **C:** Spatial working memory was investigated by measuring spontaneous alternations in a Y maze for tau^{+/+} (n = 18), tau^{-/-} (n = 8), APP/PS1/tau^{+/+} (n = 9), and APP/PS1/tau^{-/-} (n = 9) mice at 6 months of age. The number of alternations differs by genotype (APP/PS1 by tau interaction, P < 0.05, two-way analysis of variance). The APP/PS1/tau^{+/+} mice show significantly lower spontaneous alternations than tau^{+/+} mice and APP/PS1/tau^{-/-} mice (*P < 0.05, Bonferroni post hoc test).

duced survival (eg, 25% mortality at 5 months) compared to tau^{+/+} mice. APP/PS1/tau^{+/+} mice were observed to have a higher frequency of sudden death and occasional convulsive seizures. Survival of tau^{-/-} mice, although showing a tendency to decrease after 10 months, was not significantly different from the other genotypes, as reported previously.^{3,9} The evolution of body weight was also analyzed from 3 months to 12 months in the four genotypes. Body weights were not different at 3 months between the different genotypes, but APP/PS1/tau^{+/+} mice had significantly lower body weights than APP/PS1/tau^{-/-} mice at 6, 9, and 12 months (P < 0.05, by one-way-analysis of variance) (data not shown).

Motor Deficits and Working Memory Deficits in APP/PS1/tau^{+/+} Mice Are Rescued in APP/PS1/tau^{-/-} Mice

APP/PS1/tau^{+/+} mice showed a significant motor deficit by rotarod testing at 12 months of age, by comparison with wild-type and tau^{-/-} mice (Figure 3B): the time spent by APP/PS1/tau^{+/+} mice on the rotating rod before falling was 50% of the time spent by tau^{+/+} and tau^{-/-} mice. By contrast, the time spent by APP/PS1/tau^{-/-} mice was significantly higher than for APP/PS1/tau^{+/+} mice and not different from tau^{+/+} and tau^{-/-} mice.

Working spatial memory was studied by assessing spontaneous alternations in Y maze in 6 month-old mice (Figure 3C). At this age, APP/PS1/tau^{+/+} mice had no motor deficits, and their exploratory activity, as measured by the number of arm entries, was similar to that in other genotypes (data not shown; not significant by two-way analysis of variance). APP/PS1/tau^{+/+} mice, however,

Figure 4. APP/PS1/tau^{+/+} mice, but not APP/PS1/tau^{-/-} mice, show a neuronal loss and a decreased spine density. **A–D:** Cresyl violet staining of sector 1 of Ammon's horn in the hippocampus (A and C) and of the anterior horn of spinal cord (B and D) of APP/PS1/tau^{+/+} (A and B) and APP/PS1/tau^{-/-} (C and D) mice at 12 months of age. **E:** Stereological quantification of neurons in the Ammon's horn of hippocampus of tau^{+/+} (n = 4), tau^{-/-} (n = 4), APP/PS1/tau^{+/+} (n = 4), and APP/PS1/tau^{-/-} mice (n = 3). APP/PS1/tau^{+/+} mice, but not APP/PS1/tau^{-/-} mice, show a neuronal loss compared to tau^{+/+} mice. *P < 0.05, Bonferroni post hoc test, two-way analysis of variance. **F:** Stereological quantification of neurons in the anterior horn of spinal cord of tau^{+/+} (n = 3), tau^{-/-} (n = 7), APP/PS1/tau^{+/+} (n = 3), and APP/PS1/tau^{-/-} mice (n = 4). The neuronal density in the spinal cord differs by genotype (APP/PS1 by tau interaction, P < 0.01, two-way analysis of variance), and APP/PS1/tau^{+/+} mice have a lower neuronal density compared to all other genotypes. *P < 0.05, Bonferroni post hoc test. **G:** Quantification of spine density in cortical neurons of tau^{+/+} (n = 5), tau^{-/-} (n = 5), APP/PS1/tau^{+/+} (n = 4), and APP/PS1/tau^{-/-} mice (n = 4). APP/PS1/tau^{+/+} mice, but not APP/PS1/tau^{-/-} mice, show a decreased spine density compared to tau^{+/+} mice. *P < 0.05, Bonferroni post hoc test, two-way analysis of variance. **H:** Mean number of spines along 30-μm (bin size) segments of apical dendrite in the four genotypes.

Figure 5. Amyloid plaque burden is lower in *APP/PS1/tau^{-/-}* than in *APP/PS1/tau^{+/+}* mice. **A–F:** Immunolabeling of tissue sections of *APP/PS1/tau^{+/+}* (**A** and **D**) and *APP/PS1/tau^{-/-}* (**B** and **E**) 6-month-old mice with an antibody to Aβ40. The Aβ40 plaque load in the cortex (**C**) and the spinal cord (**F**) is significantly lower in *APP/PS1/tau^{-/-}* mice ($n = 5$) compared to *APP/PS1/tau^{+/+}* mice ($n = 3$). **G–L:** Immunolabeling of tissue sections of *APP/PS1/tau^{+/+}* (**G** and **J**) and *APP/PS1/tau^{-/-}* (**H** and **K**) 6-month-old mice with an antibody to Aβ42. The Aβ42 plaque load in the cortex (**I**) and the spinal cord (**L**) is significantly lower in *APP/PS1/tau^{-/-}* mice ($n = 5$) compared to *APP/PS1/tau^{+/+}* mice ($n = 3$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by Student's t -test. CC, corpus callosum; Cx, cortex; H, hippocampus; Th, thalamus. Scale bars: 200 μ m (**A**, **B**, **G**, and **H**); 100 μ m (**D**, **E**, **J**, and **K**).

showed a significant reduction in spontaneous alternations by comparison with *tau^{+/+}* mice. Spontaneous alternations were significantly higher in *APP/PS1/tau^{-/-}* mice than in *APP/PS1/tau^{+/+}* mice and not different from *tau^{+/+}* and *tau^{-/-}* mice.

Loss of Neurons and Synaptic Loss in *APP/PS1/tau^{+/+}* Mice Is Rescued in *APP/PS1/tau^{-/-}* Mice

Neuronal loss in the hippocampus and the spinal cord was then assessed by stereology with the optical fractionator method in 12-month-old mice (Figure 4). The number of neurons in Ammon's horn of the hippocampus of *APP/PS1/tau^{+/+}* mice, but not of *APP/PS1/tau^{-/-}* mice, was significantly reduced by comparison with *tau^{+/+}* (Figure 4E). In the anterior horn of spinal cord (Figure 4F), the density of neurons in *APP/PS1/tau^{+/+}* mice was reduced by 50% in comparison with *tau^{+/+}* or *tau^{-/-}* mice and was significantly higher in *APP/PS1/tau^{-/-}* mice.

We assessed the synaptic loss in *APP/PS1/tau^{+/+}* and *APP/PS1/tau^{-/-}* mice by investigating by Western blot-

ting the levels of the presynaptic marker synaptophysin and the postsynaptic marker PSD95 in 12-month-old mice. Mean brain synaptophysin and PSD95 levels were lower in *APP/PS1/tau^{+/+}* and *APP/PS1/tau^{-/-}* mice than in *tau^{+/+}* mice, but were not different between them (data not shown). We next analyzed the density of dendritic spines in Golgi-stained brain sections in 12-month-old mice. The mean spine density along a 150- μ m segment of primary apical dendrites of cortical neurons of *APP/PS1/tau^{+/+}* mice, but not of *APP/PS1/tau^{-/-}* mice, was significantly lower than in *tau^{+/+}* mice (Figure 4G). In the first 30- μ m segment, *APP/PS1/tau^{+/+}* mice had a spine density significantly reduced in comparison with all other genotypes (Figure 4H).

Amyloid Plaque Burden Is Lower in *APP/PS1/tau^{-/-}* Than in *APP/PS1/tau^{+/+}* Mice

As reported previously,¹³ amyloid plaques were first detected with anti-Aβ antibodies in young 2-month-old *APP/PS1/tau^{+/+}* mice in the subiculum and in deep cortical

Figure 6. Levels of soluble and insoluble A β are lower in *APP/PS1/tau*^{-/-} mice than in *APP/PS1/tau*^{+/+} mice. Analysis of A β in brain homogenates of *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice at 6 months of age. **A–E:** Analysis of A β levels in RIPA-soluble fraction by Western blot (*n* = 7 for each genotype) with the 6E10 antibody (**A** and **B**), and by ELISA (*n* = 3 for each genotype) for A β 40 (**C**), for A β 42 (**D**), and for the A β 42/A β 40 ratio (**E**). **F–J:** Analysis of A β levels in RIPA-insoluble fraction by Western blot (*n* = 7 for each genotype) with the 6E10 antibody (**F** and **G**), and by ELISA (*n* = 3 for each genotype) for A β 40 (**H**), for A β 42 (**I**), and for the A β 42/A β 40 ratio (**J**). A significant decrease of A β 40 and A β 42 is observed in the soluble and insoluble fractions in *APP/PS1/tau*^{-/-} mice compared to *APP/PS1/tau*^{+/+} mice. The A β 42/40 ratio is significantly decreased in the soluble fraction and is generally decreased in the insoluble fraction in *APP/PS1/tau*^{-/-} mice. Molecular weight markers: (**A** and **F**) 4 kDa (insulin β chain), 7 kDa (aprotinin), 50 kDa (alcohol dehydrogenase). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 by Student's *t*-test.

layers, and progressed later in all cortical layers, in subcortical areas, and in the brainstem. Amyloid plaques were also detected in the spinal cord. Amyloid deposition tended to be lower in male than in female mice. A similar spatial pattern of amyloid deposition in the brain was observed in *APP/PS1/tau*^{-/-} mice. Amyloid plaques, however, appeared less numerous in *APP/PS1/tau*^{-/-} mice at all investigated ages. The load of amyloid plaques in 6-month-old *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice was then quantified by immunocytochemistry with antibodies to A β 40 and to A β 42 (Figure 5). The mean area covered by A β 40-positive (Figure 5, A–F) and A β 42-positive (Figure 5, G–L) plaques was significantly lower in the cortex and the spinal cord of *APP/PS1/tau*^{-/-} mice. We then calculated the density and the mean size of A β 42- and A β 40-positive plaques in the cortex of 6-month-old mice. In *APP/PS1/tau*^{-/-} mice, the density of A β 42-positive (91 $\pm$ 16 plaques/mm²) and A β 40-positive (81 $\pm$ 14 plaques/mm²) plaques was significantly lower in comparison with *APP/PS1/tau*^{+/+} mice (157 $\pm$ 17 plaques/mm² for A β 42; 159 $\pm$ 7 plaques/mm² for A β 40) (*P* < 0.01 for A β 40 and *P* < 0.05 for A β 42, by Student's

t-test). The mean size of A β 42-positive plaques was also significantly lower in *APP/PS1/tau*^{-/-} mice (433 $\pm$ 10 μ m²) in comparison with *APP/PS1/tau*^{+/+} mice (528 $\pm$ 35 μ m²) (*P* < 0.05 by Student's *t*-test). The reduction of amyloid plaque burden in *APP/PS1/tau*^{-/-} mice was similarly observed when using Congo Red or thioflavin staining, indicating that this decrease did not result from a shift from fibrillar to diffuse A β deposition.

Levels of Soluble and Insoluble A β Are Lower in *APP/PS1/tau*^{-/-} Mice Than in *APP/PS1/tau*^{+/+} Mice

We next analyzed the levels of A β in the brain and spinal cord of 6-month-old *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice by immunoblotting with the 6E10 anti-A β antibody (recognizing both A β 40 and A β 42) (Figure 6). The levels of A β were analyzed in the RIPA soluble fractions (Figure 6, A–E) and in the RIPA insoluble fractions after formic acid extraction (Figure 6, F–J). The levels of

total A β in both the brain and the spinal cord were significantly lower in the soluble (Figure 6B) and insoluble (Figure 6G) fractions in *APP/PS1/tau*^{-/-} mice, in comparison with *APP/PS1/tau*^{+/+} mice. To determine whether this reduction concerned shorter and longer forms of A β , the levels of A β 38, A β 40, and A β 42 were then analyzed separately by enzyme-linked immunosorbent assay (ELISA) in the same fractions. By ELISA, A β 38 (data not shown), A β 40, and A β 42 levels were significantly lower in the brain and the spinal cord in the soluble (Figure 6, C and D) and insoluble (Figure 6, H and I) fractions in *APP/PS1/tau*^{-/-} mice compared to *APP/PS1/tau*^{+/+} mice. Levels of A β 42 were higher than levels of A β 40 and A β 38, both in the soluble and in the insoluble fractions in both genotypes.

Figure 7. Levels of APP β -CTFs and of phosphorylated Thr668 APP are lower in *APP/PS1/tau*^{-/-} than in *APP/PS1/tau*^{+/+} mice. **A:** Western blot analysis of brain homogenates of *tau*^{+/+}, *tau*^{-/-}, *APP/PS1/tau*^{+/+}, and *APP/PS1/tau*^{-/-} mice at 6 months of age with the BR15 antibody recognizing APP holoprotein, with the APP668P antibody recognizing APP holoprotein and CTFs phosphorylated on Thr668, and with the 6E10 antibody recognizing β -CTFs. Molecular weight markers: 16 kDa (lysozyme), 98 kDa (bovine serum albumin). **B–E:** Densitometry analysis of levels of APP, pThr668 APP, and CTFs. Levels of APP holoprotein are similar in *APP/PS1/tau*^{+/+} ($n = 9$) and *APP/PS1/tau*^{-/-} ($n = 10$) mice (**B**), but levels of pThr668 APP (**C**), of β -CTFs (**D**), and of pThr668 CTFs (**E**) are lower in *APP/PS1/tau*^{-/-} mice. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by Student's t -test.

Figure 8. Levels of BACE1 are reduced in *APP/PS1/tau*^{-/-} mice. **A:** Western blots of brain homogenates of *tau*^{+/+} (lane 1), *tau*^{-/-} (lane 2), *APP/PS1/tau*^{+/+} (lanes 3–5), and *APP/PS1/tau*^{-/-} mice (lanes 6–8) at 6 months of age with BACE1 and actin antibodies. BACE1 migrates as a 70-kDa main band. **B:** Densitometry analysis of BACE1 levels ($n = 7$ for each genotype). BACE1 levels differ by genotypes (*APP/PS1* by *tau* interaction, $P < 0.01$, two-way analysis of variance) and are significantly higher in *APP/PS1/tau*^{+/+} mice compared to other genotypes. ** $P < 0.01$, Bonferroni post hoc test. **C and D:** Double immunohistochemical labeling (cortex) with the anti-BACE1 antibody (green) and the anti-A β antibody (red) in an *APP/PS1/tau*^{+/+} (**C**) and an *APP/PS1/tau*^{-/-} (**D**) mouse. A BACE1-positive annulus surrounds the amyloid core of plaques. Scale bar = 15 μ m.

Because an increased ratio of A β 42 to A β 40 favors the aggregation of A β , we determined the A β 42/A β 40 ratios based on levels determined by ELISA in *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice. A significant decrease of the A β 42/A β 40 ratio was observed in the soluble fraction (Figure 6E) in the brain and the spinal cord of *APP/PS1/tau*^{-/-} mice. A similar tendency was also observed in the insoluble fraction (Figure 6J).

Levels of APP β -C-Terminal Fragments and of BACE1 Are Decreased in *APP/PS1/tau*^{-/-} Mice

Because a reduction of A β might result from reduced β -secretase cleavage of APP by BACE1, the levels of β -C-terminal fragments (β -CTFs) of APP were analyzed in the brain and spinal cord of 6-month-old *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice by immunoblotting (Figure 7A). The steady-state levels of β -CTFs normalized to APP holoprotein were significantly reduced in the brains (Figure 7D) and in the spinal cords (not shown) of *APP/PS1/tau*^{-/-} mice. APP phosphorylation at Thr668 has been reported to increase A β generation.^{20,24} The levels of phosphorylated APP holoprotein (Figure 7C) and of phosphorylated CTFs (Figure 7E) normalized to total APP holoprotein were significantly reduced in the brains and in the spinal cord of *APP/PS1/tau*^{-/-} mice.

BACE1 levels have been reported to be increased in 5xFAD mice.²⁵ We analyzed the levels of BACE1 in the brain of *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice by Western blotting: BACE1 levels were significantly lower in *APP/PS1/tau*^{-/-} mice (Figure 8, A and B). As reported earlier, we observed that BACE1 immunoreactivity was increased in a positive annulus surrounding the amyloid

Figure 9. pTyr216 GSK-3 β and PS1/GSK-3 β , PS1/APP-CTFs complexes are less abundant in APP/PS1/tau^{-/-} mice. **A:** Western blot analysis of brain homogenates of APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} mice with the anti-pTyr216 GSK-3 β , anti-pSer9 GSK-3 β and anti-GSK-3 β (TPK1) antibodies in 12-month-old mice. **B:** Densitometry analysis ($n = 7$ for each genotype) of levels of pTyr216 GSK-3 β and pSer9 GSK-3 β normalized to GSK-3 β . Levels of pTyr216 GSK-3 β are significantly reduced in APP/PS1/tau^{-/-} mice. **C:** PS1 was immunoprecipitated from brain homogenates (input) of APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} mice, and immunoprecipitates (IP-PS1) were analyzed by Western blotting with the PS1 antibody (SB129), the GSK-3 β antibody (TPK1), and the APP antibody (C17). The arrows point to PS1 holoprotein and N-terminal fragments, respectively. **D** and **E:** Densitometry analysis ($n = 3$ for each genotype) of levels of GSK-3 β (**D**) and APP-CTFs (**E**) in PS1 immunoprecipitates. GSK-3 β and APP-CTFs are co-immunoprecipitated with PS1 but are less abundant in APP/PS1/tau^{-/-} mice. * $P < 0.05$ by Student's t -test.

core of plaques in both APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} mice (Figure 8, C and D).²⁵ Finally, because reduction of β -secretase cleavage of APP might be associated with a relatively increased α -secretase cleavage, we calculated the ratio of α -CTFs (resulting from α -secretase cleavage) to β -CTFs (using the BR15 anti-Cter APP antibody): this ratio was slightly increased from 1.00 ± 0.06 to 1.14 ± 0.07 when comparing APP/PS1/tau^{+/+} to APP/PS1/tau^{-/-} mice. The levels of A β protease insulin-degrading enzyme and transthyretin were unchanged in APP/PS1/tau^{-/-} mice (data not shown).

Levels of PhosphoY216 GSK-3 β and of PS1/GSK-3 β /APP-CTF Complexes Are Lower in APP/PS1/tau^{-/-} Mice

GSK-3 β activity might be modulated by PS1 and has been involved in APP processing. Total levels of GSK-3 β

and of inactive pSer9 GSK-3 β were unchanged in APP/PS1/tau^{-/-} mice, but levels of active pTyr216 GSK-3 β (normalized to total GSK-3 β) were significantly reduced in these mice (Figure 9, A and B). PS1 forms a complex with GSK-3 β ¹¹ and APP, and we analyzed these complexes after immunoprecipitation of PS1 from brain homogenates of APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} mice (Figure 9C). GSK-3 β was present in PS1 immunoprecipitates in both genotypes but was reduced in APP/PS1/tau^{-/-} mice (Figure 9D). β -CTFs were also immunoprecipitated with PS1 and their levels significantly reduced in APP/PS1/tau^{-/-} mice (Figure 9E).

APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} Mice Both Develop Neuritic Plaques and a Glial Reaction

We next compared the abnormal neuritic components associated with amyloid deposits in both genotypes by immunocytochemistry with antibodies to cathepsin B and by transmission electron microscopy. Cathepsins are lysosomal proteases that have been observed to be concentrated in autophagolysosomes accumulating in dystrophic neurites in senile plaques in Alzheimer disease.²⁶ In both APP/PS1/tau^{+/+} and APP/PS1/tau^{-/-} 6-month-old mice, the vast majority of amyloid deposits were similarly associated with cathepsin B-positive dystrophic neurites, indicating that tau expression was not necessarily for the formation of these abnormal neurites (see Supplemental Figure S1, A and B, at <http://ajp.amjpathol.org>). In electron microscopy (see Supplemental Figure S1, C and D, at <http://ajp.amjpathol.org>), dystrophic neurites showed in both genotypes accumulations of autophagic vacuoles, multivesicular bodies, and mitochondria, as reported in other APP/PS1 mice.²³ We also investigated the astroglial and microglial reaction in the cortex, the hippocampus, and the spinal cord by immunohistochemistry with anti-GFAP and anti-Iba1 antibodies. Reactive astrocytes and microglial cells were similarly associated with amyloid deposits in both genotypes (data not shown).

Discussion

To assess the effect of tau deletion on the deficient phenotype induced by mutant APP and PS1, we generated a new transgenic model expressing both mutant APP and PS1 proteins, in absence of any tau proteins. APP/PS1/tau^{+/+} mice showed a deficit in working memory at 6 months of age, and we observed a complete rescue of this cognitive deficit in APP/PS1/tau^{-/-} mice. APP/PS1/tau^{+/+} mice had a reduced survival compared to tau^{+/+} mice, and this reduced survival was not observed in APP/PS1/tau^{-/-} mice. Aged APP/PS1 mice also had a motor deficit that was completely rescued in APP/PS1/tau^{-/-} mice. Thus tau deletion is effective in rescuing, not only reduced survival and cognitive deficits, but also motor deficits. A rescue of cognitive deficits by tau deletion was also observed previously in two models expressing mutant APP alone and deleted for tau,^{3,4,9} but not in

all.¹⁰ This effect of *tau* deletion on several kinds of deficient behavioral phenotypes must result from an effect targeting different neuronal populations, and suggests a more general protective effect of tau deletion than previously suggested.

Aged *APP/PS1/tau*^{+/+} (5xFAD) mice have been reported to have a loss of large pyramidal cortical neurons^{13,27}; by using an unbiased stereological method, we observed that these aged *APP/PS1/tau*^{+/+} mice also have a loss of pyramidal neurons in the hippocampus. In addition, we document a loss of neurons in the anterior spinal cord, probably linked to the development of the motor deficits in the aged *APP/PS1/tau*^{+/+} mice. Interestingly, we observed that *tau* deletion rescued this loss of neurons in the hippocampus and decreased the loss in the spinal cord. In previous studies in mice expressing only mutant APP, *tau* deletion was found to protect against early mortality, excitotoxicity, and spontaneous epileptiform activity,^{3,4,9} but an effect on neuronal death was not demonstrated: one study³ used hAPPJ20 mice that do not show substantial neuronal loss,²⁸ and the other study⁴ did not assess changes in neuronal loss using APP23 mice.²⁹ Spine loss in *APP/PS1/tau*^{+/+} mice was also partially rescued in *APP/PS1/tau*^{-/-} mice.

An interesting finding of this study is that *tau* deletion also decreases amyloid burden in *APP/PS1* mice. This decrease, roughly of 50%, concerns both the extent of extracellular deposition of A β in amyloid plaques as estimated by morphological measurements, and the levels of soluble and insoluble A β as estimated by immunoblotting and ELISA measurements. A β reduction did not result from reduced expression of mutant APP or PS1 in *APP/PS1/tau*^{-/-} mice because APP and PS1 levels were similar to those seen in *APP/PS1/tau*^{+/+} mice. Also, the levels of A β protease insulin-degrading enzyme and transthyretin were unchanged in *APP/PS1/tau*^{-/-} mice. A β reduction in *APP/PS1/tau*^{-/-} mice, however, was associated with a reduction of steady-state levels of β -CTFs. APP phosphorylation at Thr668 has been reported to facilitate BACE1 cleavage,²⁴ and β -CTF phosphorylation was reported to facilitate γ -secretase cleavage.²⁰ Levels of phosphorylated APP and β -CTFs were reduced in *APP/PS1/tau*^{-/-} mice. Also, levels of BACE1 were reduced in the *APP/PS1/tau*^{-/-} mice compared to *APP/PS1/tau*^{+/+} mice in which an increase in BACE1 levels was previously observed.²⁵ All together, this suggests that tau deletion impaired β -secretase cleavage of APP in *APP/PS1/tau*^{-/-} mice. A modification of BACE1 accessibility to APP at the plasma membrane in lipid rafts or in early endosomes and/or trafficking of BACE1 between these two compartments might conceivably be disturbed by *tau* deletion affecting actin/clathrin-mediated endocytosis. *tau* deletion was also recently reported to affect APP trafficking to the neuronal surface.³⁰

We observed a significant decrease of the active form of GSK-3 β (pY216) in *APP/PS1/tau*^{-/-} mice that might have played a role in reduction of A β levels, because a reduction of GSK-3 α ³¹ and GSK-3 β ³² activities were reported to reduce A β levels in APP mice, although the role of GSK-3 in modulation of APP processing is still a matter of investigation.^{33,34} Con-

versely, A β has been reported to induce GSK-3 β activation,^{7,35} and reduction of A β levels might also reduces GSK-3 β activation on its own.

We also observed a reduced association of GSK-3 β with PS1 in *APP/PS1/tau*^{-/-} mice. Because GSK-3 β -mediated phosphorylation of PS1 inhibits the PI3/AKT activation of cell survival signaling,³⁶ reduction of GSK-3 β -PS1 association in *APP/PS1/tau*^{-/-} mice could reduce the PS1-mediated inhibition of cell survival, rescuing neuronal cell death observed in these mice. *tau* deletion was also reported to reduce GSK-3 β -induced hippocampal neurodegeneration.³⁷

In addition to a reduction of A β , *tau* deletion in *APP/PS1/tau*^{-/-} mice led to a decrease of the A β 42/A β 40 ratio in comparison with *APP/PS1* mice. The *APP/PS1* (5xFAD) model used in this study expresses a mutant APP bearing three FAD mutations (Swedish, Florida, and London), and a mutant PS1 bearing two FAD mutations. The Swedish mutation increases the production of total A β , and the Florida, the London, and the PS1 mutations increase the production of A β 42. The combination of these mutations is additive and leads to the production of high levels of A β 42.¹³ High A β 42/A β 40 ratios promote plaque nidus formation, which are more numerous in *APP/PS1* (5xFAD) mice than in APP mice, such as Tg2576 expressing only the APPsw mutation.¹³ The lower density of amyloid plaques and the smaller size of A β 42-positive plaques in *APP/PS1/tau*^{-/-} mice is compatible with less nucleation nidus and slower growth due to a reduced A β 42/A β 40 ratio. Interestingly, although *tau* deletion reduced the amyloid load, amyloid deposits were systematically associated with dystrophic neurites in both *APP/PS1/tau*^{+/+} and *APP/PS1/tau*^{-/-} mice, suggesting that tau expression was not necessarily for the formation of these abnormal processes.

Like other APP transgenic lines,^{3,4} *APP/PS1/tau*^{+/+} mice in our study exhibited early mortality and a higher frequency of sudden death. Many mouse AD models exhibit epileptic activity that might explain reported sudden death in APP mice.^{38,39} Tau reduction might reduce this mortality by mechanisms associated with reduced sensitivity to excitotoxicity, but also by reducing A β load as observed in the present study.

Improved survival of *APP/PS1/tau*^{-/-} mice was paralleled by rescue of motor deficit, neuronal loss, decreased spine density, and by decreased GSK-3 β activity, compared to *APP/PS1/tau*^{+/+} mice. These improved biological parameters might increase survival by themselves. Conversely, improved survival, eg, due to less A β load and toxicity, could also improve these biological parameters.

A β levels and amyloid plaque burden were not observed to be affected in mice expressing only mutant APP^{28,29} crossed with *tau*^{-/-} mice,^{3,4} although A β levels were observed to be increased in another study.¹⁰ *tau*^{-/-} mice in these studies were of different origin,^{14,40} but we used the same *tau*^{-/-} line¹⁴ as in one of the previous studies,⁴ suggesting that our observation of decreased amyloid burden in *APP/PS1* mice was not due to the use of a different genetic background associated with *tau* deletion, but rather to the presence of a mutant PS1. A

modification of APP processing induced by *tau* deletion could also conceivably result from changes in the interaction between the two proteins. APP and tau have been reported to interact with each other.⁴¹ Some antibodies to APP have been reported to label neurofibrillary tangles and isolated paired helical filaments,^{42–46} and tau enhances the formation of amyloid fibrils.⁴⁷

Thus, *tau* deletion might prevent A β toxicity by several mechanisms. As reported previously in APP mice, this includes control of neuronal excitability, alterations in Fyn subcellular localization, absence of generation of tau neurotoxic fragments, A β -induced deficits in axonal transport, and decreased microtubule stability.^{48,49} Here, we report an additional mechanism in *APP/PS1* mice, in which *tau* deletion was found to reduce amyloidogenic processing of APP.

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