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Seizure Resistance without Parkinsonism in Aged Mice after Tau Reduction

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Abstract

Tau is an emerging target for Alzheimer disease and other conditions with epileptiform activity. Genetic tau reduction (in *Tau*^{+/-} and *Tau*^{-/-} mice) prevents deficits in Alzheimer disease models and has an excitoprotective effect, increasing resistance to seizures, without causing apparent neuronal dysfunction. However, most studies of tau reduction have been conducted in mice less than one year old and the effects of tau reduction in aged mice are less clear. Specifically, whether the excitoprotective effects of tau reduction persist with aging is unknown and whether tau reduction causes neuronal dysfunction, including parkinsonism, with aging is controversial. Here we performed a comprehensive analysis of two-year-old *Tau*^{+/+}, *Tau*^{+/-}, and *Tau*^{-/-} mice. In aged mice, tau reduction still conferred resistance to pentylentetrazole-induced seizures. Moreover, tau reduction did not cause parkinsonian abnormalities in dopamine levels or motor function and did not cause iron accumulation or impaired cognition, although *Tau*^{-/-} mice had mild hyperactivity and decreased brain weight. Importantly, the excitoprotective effect in aged *Tau*^{+/-} mice was not accompanied by detectable abnormalities, indicating that partially reducing tau or blocking its function may be a safe and effective therapeutic approach for Alzheimer disease and other conditions with increased excitability.

1. Introduction

The microtubule-associated protein tau plays an important role in the pathogenesis of Alzheimer disease (AD). Genetically reducing levels of tau, either partially in *Tau*^{+/-} mice or completely in *Tau*^{-/-} mice, prevents impairment in learning and memory, epileptiform activity and other AD-related deficits in multiple mouse models of AD (Roberson et al., 2007; Palop et al., 2007; Meilandt et al., 2008; Ittner et al., 2010; Andrews-Zwilling et al., 2010; Roberson et al., 2011; Nussbaum et al., 2012; Leroy et al., 2012). Tau reduction also has an excitoprotective effect in pharmacological and genetic models of epilepsy (Roberson

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et al., 2007; Ittner et al., 2010; Holth et al., 2013; Devos et al., 2013). Notably, even partial tau reduction is excitoprotective (Roberson et al., 2007; Ittner et al., 2010; Holth et al., 2013; Devos et al., 2013). Therefore, tau reduction has been proposed as a treatment strategy for AD and other conditions with epileptiform activity.

While tau reduction is beneficial and does not cause major neuronal dysfunction in mice up to one year of age (Dawson et al., 2001; Tucker et al., 2001; Roberson et al., 2007; Lei et al., 2012), the long-term effects of tau reduction have been less well studied. It is unclear whether the beneficial effects of tau reduction persist and whether long-term tau reduction has detrimental effects with aging. In fact, *Tau*^{-/-} mice were recently reported to develop parkinsonism after one year, including impaired motor coordination, reduced dopamine levels, and neuronal loss related to iron accumulation (Lei et al., 2012). However, others have been unable to reproduce these findings (Morris et al., 2013), and it is unknown whether the previously described excitoprotective effects persist with aging. To address these questions regarding the long-term effects of tau reduction, we performed a comprehensive analysis of two-year-old *Tau*^{+/+}, *Tau*^{+/-} and *Tau*^{-/-} mice.

2. Methods

2.1 Animals

The tau knockout mice were originally developed by Dawson et al (2001). The mice used here were on a congenic C57BL/6J background. Heterozygous tau knockout mice (*Tau*^{+/-}) were bred to generate littermate *Tau*^{+/+}, *Tau*^{+/-} and *Tau*^{-/-} mice. Mice were then aged to around 2 years. As in younger mice, aged *Tau*^{+/-} mice had tau protein levels about 50% of wild-type *Tau*^{+/+} mice while *Tau*^{-/-} mice had no detectable tau protein by western blot (Figure S1).

Both male and female mice were used in experiments and no sex-dependent effects were observed. Mice were housed in a pathogen-free barrier facility on a 12-hour light/dark cycle with *ad libitum* access to water and food (NIH-31 Open Formula Diet, #7917, Harlan). For postmortem analyses, mice were anesthetized by Fatal-plus (Vortech) and perfused with 0.9% saline. Brains were then removed, weighed, and dissected for processing as described below. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

2.2 Pentylentetrazole-induced seizures

Pentylentetrazole (PTZ)-induced seizure susceptibility was measured as previously described (Roberson et al., 2011). Briefly, PTZ (Sigma) was dissolved in PBS at a concentration of 4 mg/ml. PTZ at dose of 40 mg/kg body weight was injected intraperitoneally. Immediately after PTZ injection, each mouse was placed into a cage for 20 minutes and observed by an investigator blind to its genotype, with video recording. The following scale of seizure stages was used: 0, normal behavior; 1, immobility; 2, generalized spasm, tremble, or twitch; 3, tail extension; 4, forelimb clonus; 5, generalized clonic activity; 6, bouncing or running seizures; 7, full tonic extension; 8, death (Racine, 1972; Loscher et al., 1991). The latency to reach each stage and the maximum stage each mouse reached were recorded.

2.3 Dopamine measurement

Immediately after sacrifice and perfusion, the striatum was dissected and a punch was removed using a Pasteur pipette. The sample was flash frozen on dry ice and dopamine levels were determined by high-performance liquid chromatography at the Vanderbilt Neurochemistry Core Lab.

2.4 Behavioral tests

All behavioral testing was carried out during the light cycle, at least one hour after the lights came on. Mice were transferred to the testing room for acclimation at least one hour prior to experiments. Testing apparatuses were cleaned with 75% ethanol between experiments and disinfected by 2% chlorhexidine after experiments were finished each day. All mice were tested in all the behavioral tests in the same order. Investigators were blind to the genotype of individual mouse at the time of experiments.

2.4.1 Rotarod—Mice were placed onto an accelerating rod (4–40 rpm over 5 min; Med Associates). The duration that each mouse remained on the rod was recorded. Each mouse was tested 5 times with at least 4 hours between each test and the average of 5 trials was reported.

2.4.2 Pole test—A wooden pole (3/8 inch diameter, 2 feet long) was placed perpendicular to the ground in a cage with 1-inch deep bedding. Rubber bands were wrapped around the pole every 1.5 inches to provide grip. Each mouse was placed on the top of pole facing downwards and then released. All mice were trained for five trials on the first day and then tested with 5 consecutive trials on the next day. The amount of time each mouse needed to walk down into the cage was recorded and the best (shortest) trial was reported.

2.4.3 Beam walk test—A 1-yard long narrow beam was placed on two stands, with one end 15 cm off the ground and the other end 20 cm off the ground. A bright light was placed close to the lower end of beam, and a container with food pellets was placed at the higher end. The ceiling lights in the testing room were turned off during testing. When put at the lower end that is close to the bright light, a mouse usually walks as fast as it can to the other end of the beam. Training started with three consecutive trials on a 1.25-inch diameter beam, followed a week later by three consecutive trials on a 1-inch diameter beam. Testing was conducted a week after the end of training and consisted of three consecutive trials on a 0.75-inch diameter beam. The time it took each mouse to cross the beam was measured based on recorded video. The average of the three trials on the 0.75-inch beam was reported.

2.4.4 Open field—Each mouse was placed into the corner of an open field apparatus (Med Associates) and allowed to walk freely for 15 minutes. Minute-by-minute ambulatory distance and rearing (vertical counts) of each mouse were determined using the manufacturer's software. Average velocity of each mouse was calculated by dividing total ambulatory distance by total ambulatory time.

2.4.5 Y-Maze—The Y-Maze apparatus consisted of three 15-inch long, 3.5-inch wide and 5-inch high arms made of white opaque plexiglass placed on a table. Each mouse was placed

into the hub and allowed to freely explore for 6 minutes, with video recording. An entry was defined as the center of the mouse's body extending 2 inch into an arm, using tracking software (CleverSys). The chronological order of entries into respective arms was determined. Each time the mouse entered all three arms successively (e.g. A-B-C or A-C-B) was considered a set. Percent alternation was calculated by dividing the number of sets by the total number of entries minus two (since by definition, the first two entries cannot meet criteria for a set). Mice with 12 or fewer total entries were excluded from spontaneous alternation calculations due to insufficient sample size.

2.4.6 Contextual fear conditioning—Experiments were conducted in Quick Change Test chambers (Med Associates). For the acquisition of fear memory, each mouse was trained by the following protocol: 2 minute acclimation to the chamber, three mild foot shocks (0.5 mA, 2 seconds duration, 1 minute apart) each co-terminating with a 20-second auditory cue (white noise, 75 dB), and 3 minutes of association time after the last foot shock. For testing, each mouse was returned to the same chamber 24 hours after training and video-recorded for 5 minutes. The percentage of time freezing was calculated using the manufacturer's software. The following parameters were used to calculate the percent of freezing of each mouse: Motion Threshold, 20; Min Freeze Duration, 1s; Method, linear.

2.5 Iron measurement

A triple quadrupole ICP-MS (Agilent 8800 ICP-QQQ) instrument was utilized for its enhanced sensitivity and interference control in MS/MS mode. Samples were first digested in ultra-pure nitric acid, hydrogen peroxide and hydrochloric acid, and then diluted to volume with deionized water. The resulting digestate was then introduced to the instrument via a quartz concentric nebulizer and a Scott spray chamber. Standards and samples were analyzed in MS/MS mode, monitoring multiple Fe masses (m/z) to include 54, 56, and 57. In MS/MS, or mass shift mode, the precursor ion is reacted with oxygen under vacuum resulting in the formation of a product ion (i.e. $\text{Fe-54} + \text{O-16} = \text{FeO-70}$). For reporting purposes, $^{57}\text{Fe}^{16}\text{O}$ (73 m/z) was chosen as the reportable mass. Prior to analysis, a ten point calibration was performed along with QA/QC samples to verify the accuracy of the resulting curve. A minimum of one aliquot of each tissue type was analyzed in duplicate, in addition to a matrix spike, to verify analytical precision and accuracy in this sample matrix. To monitor instrument stability, a Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) was analyzed evenly in ten replicates to confirm accuracy and background. The resulting sample concentrations were then reported as μg of iron per gram of wet tissue (PPM).

2.6 Brain histology

Mouse brains were drop-fixed in 4% paraformaldehyde in phosphate buffer (80mM Na_2HPO_4 , 200mM NaH_2PO_4 , pH 7.4) for 48 hours and stored in PBS at 4°C until used. Brains were transferred into 30% sucrose for cryoprotection, then sectioned on a sliding microtome with freezing stage (Leica) into 30 μm thick sections. Brain sections were mounted onto glass with 1% gelatin and stained with cresyl violet. Sections at bregma -0.26mm were identified by the anterior commissure. Pictures of the whole section were

taken on Nikon bright field microscope with 4X objective. Quantification of regional thickness/area was performed using ImageJ.

2.7 Statistical analysis

Data are presented as mean $\pm$ standard error of the mean. Statistical analyses were performed using Prism 6.0 (GraphPad). Each dataset was evaluated for normality. Normal datasets were evaluated using ANOVA with *post hoc* Dunnett's multiple comparison test vs. wild-type. Nonparametric datasets were evaluated using the Kruskal-Wallis test and Dunn's multiple comparison test vs. wild-type. The survival data was analyzed by Kaplan-Meier statistics and post-hoc Log-rank (Mantel-Cox) test. A threshold of $p < 0.05$ was considered significant.

3. Results

3.1 Excitoprotective effects of tau reduction persist in aged mice

Aberrant excitatory neuronal activity induced by high levels of A β contributes to cognitive impairment and other deficits in mice (Palop and Mucke, 2010). Because tau reduction in young mice confers resistance to seizures induced by PTZ (Roberson et al., 2007; Ittner et al., 2010), kainate (Roberson et al., 2007; Palop et al., 2007), A β (Roberson et al., 2011), and potassium channel dysfunction (Holth et al., 2011), decreasing neuronal excitability is considered a possible mechanism by which tau reduction prevents AD-like deficits in mouse models. To determine whether these excitoprotective effects of tau reduction seen in younger mice persist in aged mice, we evaluated seizure susceptibility of $Tau^{+/+}$, $Tau^{+/-}$ and $Tau^{-/-}$ mice at 23–25 months in a large cohort of mice on a congenic C57BL/6J background.

We used the PTZ-induced seizure model. In this model, mice progress through stereotypic stages of seizure severity. The latency to each stage and the maximum stage reached are indications of the susceptibility to seizures. In young mice, tau reduction increases latency and decreases maximal seizure stage (Roberson et al., 2007; Ittner, 2010 #32545). After intraperitoneal injection of PTZ, aged $Tau^{+/-}$ and $Tau^{-/-}$ mice had longer seizure latencies than $Tau^{+/+}$ mice (Fig. 1A). Tau reduction also reduced the maximal seizure stage in aged mice (Fig. 1B). Thus, the excitoprotective effects of tau reduction previously observed in young mice persist in aged mice.

3.2 Absence of parkinsonian phenotypes in aged mice with tau reduction

Next we evaluated potential adverse effects of tau reduction with aging. There were no differences in survival into old age between $Tau^{+/+}$, $Tau^{+/-}$, and $Tau^{-/-}$ mice (Fig. 2).

A prior study reported parkinsonism (dopamine deficiency and motor deficits including decreased time remaining on accelerating rod, slower descent on the pole test and decreased distance in an open field) associated with iron accumulation in aged (> 12 months) $Tau^{-/-}$ mice (Lei et al., 2012), but these findings were not reproduced by another group (Morris et al., 2013). We measured levels of dopamine and its metabolite, homovanillic acid (HVA), in the striatum of aged mice. Striatal dopamine and HVA levels were not different from $Tau^{+/+}$

mice in aged $Tau^{+/-}$ or $Tau^{-/-}$ mice (Fig. 3). We also examined motor function using the paradigms employed by Lei et al (2012), plus beam walk which is another common test of motor function. On the accelerating rotarod, aged $Tau^{+/-}$ and $Tau^{-/-}$ mice were not different from $Tau^{+/+}$ mice (Fig. 4A). On the pole test, aged $Tau^{+/-}$ mice were not different from $Tau^{+/+}$ mice and aged $Tau^{-/-}$ mice actually descended slightly faster than $Tau^{+/+}$ mice (Fig. 4B), opposite the slower descent expected with parkinsonism. On the beam walk, aged $Tau^{+/-}$ and $Tau^{-/-}$ mice had no abnormalities in time to cross (Fig. 4C). Finally, in the open field, ambulation was not different from $Tau^{+/+}$ mice in aged $Tau^{+/-}$ and $Tau^{-/-}$ mice, while aged $Tau^{-/-}$ mice reared more than normal (Fig. 4D–F).

3.3 Age-appropriate cognitive function in aged mice with tau reduction

We next examined the long-term effects of tau reduction on cognition. We first evaluated short-term working memory using the Y maze. Because of their tendency to explore novel environments, mice freely exploring the Y maze tend to alternate between the three arms, which requires intact short-term working memory. There were no differences in percent alternation between aged $Tau^{+/+}$, $Tau^{+/-}$, and $Tau^{-/-}$ mice (Fig. 5A).

We next evaluated long-term spatial associative memory using classical fear conditioning. When a mouse enters an environment in which it previously had an aversive experience (mild foot shock), it tends to freeze as a manifestation of fear. Thus, percent time spent freezing is a gauge of spatial memory. Aged $Tau^{+/-}$ and $Tau^{-/-}$ mice exhibited fear conditioning comparable to $Tau^{+/+}$ mice, indicating no deficit in long-term associative memory (Fig. 5B).

3.4 Iron levels comparable to control in aged mice with tau reduction

Our behavioral and neurochemical results differ from those of Lei et al. (2012), who observed parkinsonism and cognitive impairment due to iron accumulation in aged $Tau^{-/-}$ mice. One difference between the studies was genetic background; our mice were congenic C57BL/6 while those of Lei et al. were on a mixed C57BL/6 and Sv129 background. C57BL/6 mice are reportedly resistant to iron overload–induced cardiotoxicity (Musumeci et al., 2013). That observation suggests two possible explanations for how the difference in background could account for the discrepancy in results: either our mice do not have iron accumulation, or they are resistant to the neurological effects of iron accumulation due to their greater proportion of C57BL/6 background. To distinguish between these possibilities, we measured total iron levels in several brain regions. There were no abnormalities in iron levels in cortex, hippocampus, or substantia nigra in aged $Tau^{+/-}$ or $Tau^{-/-}$ mice (Fig. 6). Thus, in our aged $Tau^{-/-}$ mice with littermate controls on a congenic C57BL/6 background, tau reduction does not lead to iron accumulation, which likely explains why we do not see the motor or cognitive abnormalities observed by Lei et al.

3.5 Age-independent decrease in brain weight of $Tau^{-/-}$ mice

Finally, we examined the effects of long-term tau reduction on brain size. Aged $Tau^{-/-}$ mice were reported to have decreased gross brain weight and enlarged ventricles (Lei et al., 2012). Consistent with those findings, we also observed a small (8%) but significant reduction in total brain weight in aged $Tau^{-/-}$ mice (Fig. 7A).

This was not due to a generalized size difference, as there were no differences in total body weight (Fig. 7B). Brain weight was not decreased in aged *Tau*^{+/-} mice (Fig. 7A).

We subsequently measured the size of different brain structures to better characterize this abnormality. Both cortical and subcortical (striatum) structures were smaller (84% and 89% of *Tau*^{+/+} mice, respectively) in *Tau*^{-/-} mice (Fig. 7C), consistent with Lei et al. However, we found that the lateral ventricle was also smaller in *Tau*^{-/-} mice (Fig. 7C). This suggests that the smaller size of *Tau*^{-/-} brains was not due to brain atrophy, which would increase the size of the ventricle. In fact, the area of the entire section was smaller (Fig. 7C), suggesting that perhaps the *Tau*^{-/-} brains developed slightly smaller.

To test this hypothesis, we measured brain weight in a cohort of young (2.5-month-old) mice and observed a similar small (5%) but significant decrease in brain weight in *Tau*^{-/-} mice, with no change in *Tau*^{+/-} mice (Fig 7D). Again, the decrease in brain weight in young *Tau*^{-/-} mice was independent of a body weight difference (Fig. 7E). There was no significant interaction between tau and age on brain weight ($F(2,97) = 0.9252, p = 0.44$). These results suggest that the reduced brain weight in *Tau*^{-/-} mice may be more likely a neurodevelopmental, rather than neurodegenerative, effect.

4. Discussion

The goal of this study was to determine if the excitoprotective effects of tau reduction seen in younger mice persist with aging and if they are accompanied by significant adverse effects. We found that aged (>22-month-old) *Tau*^{-/-} mice were resistant to seizures, just as younger *Tau*^{-/-} mice are. Moreover, while the aged *Tau*^{-/-} mice had mild hyperactivity as previously described in younger *Tau*^{-/-} mice, we found no behavioral or neurochemical evidence of parkinsonism and no cognitive deficits. Perhaps even more important are our findings in *Tau*^{+/-} mice with tau protein levels ~50% of *Tau*^{+/+} mice. Aged *Tau*^{+/-} mice were also resistant to seizures and did not have even the relatively minor abnormalities seen in aged *Tau*^{-/-} mice. Thus, partial tau reduction is excitoprotective without adverse effects, even over a span of almost two years, most of the lifetime of the mouse.

There have been conflicting reports on adverse effects in aged *Tau*^{-/-} mice. Lei et al (2012) found iron accumulation, dopaminergic cell loss, parkinsonian motor impairment, and Y-maze memory deficits whereas Morris et al. (2013) found only mild motor impairment with dopamine levels and cognition comparable to *Tau*^{+/+} mice. Our study, finding only mild hyperactivity in aged *Tau*^{-/-} mice but no parkinsonism or cognitive impairment, is more consistent with Morris et al. (Morris et al., 2013). The reason for the discrepancy between studies is not fully clear, since all three studies examined mice with the same tau knockout construct (Dawson et al., 2001) using many of the same tests. One reason could be the difference in genetic background, which is known to affect phenotypes in mouse models of epilepsy (Schauwecker, 2012) and parkinsonism (Boyd et al., 2007). The mice in both Morris et al. (2013) and our study were on a congenic C57BL/6J background, while those in Lei et al. (2012) were mixed C57BL/6 and 129/Sv. While C57BL/6 mice show some resistance to the cardiac effects of iron overload (Musumeci et al., 2013), this would not explain the discrepant results, as there was no evidence of iron overload in either our study

(Fig. 4) or in Morris et al. (2013). Another reason might be how the experimental and control mice were bred. The strength of both Morris et al. (2013) and our study is the strategy of breeding *Tau*^{+/-} mice to generate littermates of the three genotypes, enabling direct comparison without concerns of genetic drift between separate colonies of *Tau*^{+/+} and *Tau*^{-/-} mice.

Tau^{-/-} mice are of interest because they provide a tool for studying the effects of tau loss of function, which has been considered one potential mechanism in tauopathies, because phosphorylation and aggregation of tau limit its ability to stabilize microtubules. However, for the most part, *Tau*^{-/-} mice have shown surprisingly few differences from *Tau*^{+/+} mice, with normal survival (Roberson et al., 2007 and Fig. 2) and without abnormalities in gross brain histology by immunohistochemistry (Dawson et al., 2001; Tucker et al., 2001). No abnormalities have been reported in learning and memory on a variety of tests including radial arm maze, Morris water maze, T-maze, Y-maze, novel object, and fear conditioning (Ikegami et al., 2000; Roberson et al., 2007; Ittner et al., 2010; Roberson et al., 2011; Morris et al., 2013 and Fig. 5). One fairly consistent abnormality in *Tau*^{-/-} mice is mild motor deficits generally reflecting hyperactivity (Ikegami et al., 2000; Morris et al., 2013 and Fig. 3), although it is interesting that tau reduction actually prevents – not exacerbates – the hyperactivity seen in human amyloid precursor protein (hAPP) transgenic models of AD (Roberson et al., 2007). Whether tauopathies are due more to gain- or loss-of-tau-function remains under inquiry, but the important point to emphasize is that to whatever extent deficits in tauopathies are due to loss of function, such loss of function would be only partial, and partial loss of tau in *Tau*^{+/-} mice has not been associated with abnormalities in any of these studies, even with aging. Thus, the data from studies of *Tau*^{+/-} mice (with only partial tau reduction) does not provide strong support for the loss-of-function hypothesis.

The induction of seizure resistance by tau reduction has emerged as one of the most consistent phenotypes in tau-deficient mice. *Tau*^{-/-} and *Tau*^{+/-} mice are resistant to epileptiform activity induced by hAPP/A β (Roberson et al., 2007; Roberson et al., 2011), GABA antagonists (Roberson et al., 2007; Ittner et al., 2010), glutamate agonists (Roberson et al., 2007), and potassium channel mutations (Holth et al., 2011). Tau antisense oligonucleotides also induce seizure resistance (Devos et al., 2013). In addition, hippocampal slices from *Tau*^{-/-} mice are resistant to epileptiform bursting induced by bicuculline (Roberson et al., 2011). And the observation is not limited to mice, as tau-deficient flies were also resistant in two *Drosophila* seizure models (Holth et al., 2011). It was recently reported that both *Tau*^{+/-} and *Tau*^{-/-} mice have reduced long-term depression (but not long-term potentiation) in hippocampal area CA1 (Kimura et al., 2014); this is one of the first observations of synaptic changes in *Tau*^{+/-} mice and could provide clues to the synaptic mechanisms underlying the effects of tau reduction. Together, these findings suggest that tau plays an important role regulating neuronal excitability in the adult brain. The molecular basis of this effect, including whether it is related to or independent of tau's microtubule-binding role, is a critical goal for future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Andrews-Zwilling Y, Bien-Ly N, Xu Q, Li G, Bernardo A, Yoon SY, Zwilling D, Yan TX, Chen L, Huang Y. Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *J Neurosci*. 2010; 30:13707–13717. [PubMed: 20943911]
- Boyd JD, Jang H, Shepherd KR, Faherty C, Slack S, Jiao Y, Smeyne RJ. Response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta. *Brain Res*. 2007; 1175:107–116. [PubMed: 17884023]
- 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]
- Devos SL, Goncharoff DK, Chen G, Kebodeaux CS, Yamada K, Stewart FR, Schuler DR, Maloney SE, Wozniak DF, Rigo F, Bennett CF, Cirrito JR, Holtzman DM, Miller TM. Antisense reduction of tau in adult mice protects against seizures. *J Neurosci*. 2013; 33:12887–12897. [PubMed: 23904623]
- Holth J, Reed JG, Inoue T, Pautler R, Botas J, Noebels J. Tau loss regulates excitability in mouse and *Drosophila* genetic models of epilepsy. *Soc Neurosci Abstr* 671.08. 2011
- Holth JK, Bomben VC, Reed JG, Inoue T, Younkin L, Younkin SG, Pautler RG, Botas J, Noebels JL. Tau loss attenuates neuronal network hyperexcitability in mouse and *Drosophila* genetic models of epilepsy. *J Neurosci*. 2013; 33:1651–1659. [PubMed: 23345237]
- Ikegami S, Harada A, Hirokawa N. Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. *Neurosci Lett*. 2000; 279:129–132. [PubMed: 10688046]
- Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wölfling H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Götz J. Dendritic function of tau mediates amyloid- β toxicity in Alzheimer's disease mouse models. *Cell*. 2010; 142:387–397. [PubMed: 20655099]
- Kimura T, Whitcomb DJ, Jo J, Regan P, Piers T, Heo S, Brown C, Hashikawa T, Murayama M, Seok H, Sotiropoulos I, Kim E, Collingridge GL, Takashima A, Cho K. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos Trans R Soc Lond B Biol Sci*. 2014; 369:20130144. [PubMed: 24298146]
- Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, Wong BX, Adlard PA, Cherny RA, Lam LQ, Roberts BR, Volitakis I, Egan GF, McLean CA, Cappai R, Duce JA, Bush AI. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med*. 2012; 18:291–295. [PubMed: 22286308]
- Leroy K, Ando K, Laporte V, Dedecker R, Suain V, Authélet M, Héraud C, Pierrot N, Yilmaz Z, Octave JN, Brion JP. Lack of tau proteins rescues neuronal cell death and decreases amyloidogenic processing of APP in APP/PS1 mice. *Am J Pathol*. 2012; 181:1928–1940. [PubMed: 23026200]
- Loscher W, Honack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylentetrazole seizure models. *Epilepsy Res*. 1991; 8:171–189. [PubMed: 1907909]
- Meilandt WJ, Yu GQ, Chin J, Roberson ED, Palop JJ, Wu T, Scarce-Levie K, Mucke L. Enkephalin elevations contribute to neuronal and behavioral impairments in a transgenic mouse model of Alzheimer's disease. *J Neurosci*. 2008; 28:5007–5017. [PubMed: 18463254]

- Morris M, Hamto P, Adame A, Devidze N, Masliah E, Mucke L. Age-appropriate cognition and subtle dopamine-independent motor deficits in aged *Tau* knockout mice. *Neurobiol Aging*. 2013; 34:1523–1529. [PubMed: 23332171]
- Musumeci M, Maccari S, Sestili P, Massimi A, Corritore E, Marano G, Catalano L. The C57BL/6 genetic background confers cardioprotection in iron-overloaded mice. *Blood Transfus*. 2013; 11:88–93. [PubMed: 22790263]
- Nussbaum JM, Schilling S, Cynis H, Silva A, Swanson E, Wangsanut T, Tayler K, Wiltgen B, Hatami A, Ronicke R, Reymann K, Hutter-Paier B, Alexandru A, Jagla W, Graubner S, Glabe CG, Demuth HU, Bloom GS. Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid- β . *Nature*. 2012; 485:651–655. [PubMed: 22660329]
- Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron*. 2007; 55:697–711. [PubMed: 17785178]
- Palop JJ, Mucke L. Amyloid- β -induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci*. 2010; 13:812–818. [PubMed: 20581818]
- Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972; 32:281–294. [PubMed: 4110397]
- Roberson ED, Halabisky B, Yoo JW, Yao J, Chin J, Yan F, Wu T, Hamto P, Devidze N, Yu GQ, Palop JJ, Noebels JL, Mucke L. Amyloid- β /Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. *J Neurosci*. 2011; 31:700–711. [PubMed: 21228179]
- Roberson ED, Scarce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid β -induced deficits in an Alzheimer's disease mouse model. *Science*. 2007; 316:750–754. [PubMed: 17478722]
- Schauwecker PE. Strain differences in seizure-induced cell death following pilocarpine-induced status epilepticus. *Neurobiol Dis*. 2012; 45:297–304. [PubMed: 21878392]
- Tucker KL, Meyer M, Barde YA. Neurotrophins are required for nerve growth during development. *Nat Neurosci*. 2001; 4:29–37. [PubMed: 11135642]

Appendix

Supplemental data associated with this article can be found in the online version.

Fig. 1.

Excitoprotective effects of tau reduction persist in aged mice. 23–25-month-old mice were tested for susceptibility to PTZ-induced seizures ($N = 13$ –20 mice per genotype). (A) Latency to each stage. Tau reduction increased the latency of PTZ-induced seizures (ANOVA, tau genotype $\times$ stage interaction, $p < 0.05$; * $Tau^{+/-}$ mice differed from $Tau^{+/+}$ at stages 5–8 and $Tau^{-/-}$ mice differed from $Tau^{+/+}$ mice at stages 6–8 on *post hoc* tests, $p < 0.05$). (B) Maximal seizure stage. Tau reduction decreased the severity of seizures (Kruskal-Wallis test, $p < 0.05$; * $p < 0.05$, † $p = 0.078$ on *post hoc* tests).

Fig. 2.

Normal survival in aging tau-deficient mice. Kaplan-Meier analysis showed no differences in spontaneous mortality between aging $Tau^{+/+}$, $Tau^{+/-}$, and $Tau^{-/-}$ mice ($N = 44-97$ mice per genotype).

Fig. 3.

Normal striatal dopamine levels in aging mice with tau reduction. There were no differences in levels of (A) dopamine (DA) or (B) the end product of its metabolism, homovanillic acid (HVA) measured in the striatum of mice aged 23–25 months ($N = 10–13$ mice per genotype).

Fig. 4.

Tau reduction does not cause parkinsonism in aged mice. 22–24-month-old mice were tested for parkinsonian motor phenotypes on four tests ($N = 13$ –22 mice per genotype). (A) Rotarod. There were no differences in the duration mice remained on the accelerating rod. (B) Pole test. There were no differences in $Tau^{+/-}$ mice. $Tau^{-/-}$ mice descended the pole more quickly than $Tau^{+/+}$ controls (ANOVA, $p < 0.001$; * $p < 0.05$ on *post hoc* tests). (C) Beam walk. There were no differences in the time needed to cross. (D–F) Open field. (D) There were no differences in ambulatory distance. (E) There were no differences in the average velocity. (F) There were no abnormalities in rearing in $Tau^{+/-}$ mice, but aged $Tau^{-/-}$ mice had more rearings than $Tau^{+/+}$ controls (ANOVA, $p < 0.01$; * $p < 0.05$ on *post hoc* tests).

Fig. 5.

Normal cognitive function in aged mice with tau reduction. 22–25-month-old mice were tested for short-term working memory and long-term spatial memory. (A) Y-Maze. There were no differences in percent alternation, a measure of short-term working memory ($N = 12$ – 15 mice per genotype). (B) Contextual fear conditioning. There were no differences in percent time freezing 24 hours after training ($N = 13$ – 20 mice per genotype).

Fig. 6.

Normal levels of iron in brains of aged mice with reduced tau levels. There were no differences in total iron content in (A) cortex, (B) hippocampus, or (C) substantia nigra in 23–25-month-old mice ($N = 10$ – 11 mice per genotype).

Fig. 7.

Age-independent decrease in brain weight of $Tau^{-/-}$ mice. (A) Brain weight was decreased in aged (23–25-month) $Tau^{-/-}$ mice (ANOVA, $p < 0.005$; ** $p < 0.005$ on *post hoc* tests), but not in $Tau^{+/-}$ mice ($N = 19$ –24 mice per genotype). (B) There were no differences in body weight in aged tau-deficient mice. (C) Sizes of different regions of brain sections at Bregma 0.26mm. Cortical thickness, and the size of the striatum, lateral ventricle, and entire section were smaller in aged $Tau^{-/-}$ mice (ANOVAs: $p < 0.05$; * $p < 0.05$ on *post hoc* tests), but not in $Tau^{+/-}$ mice. ($N = 7$ –12 mice per genotype). (D) Brain weight was decreased in young (2–5-month) $Tau^{-/-}$ mice (ANOVA, $p < 0.005$; ** $p < 0.005$ on *post hoc* tests), but

not in *Tau*^{+/-} mice (*N* = 11–16 mice per genotype). (E) There were no differences in body weight in young tau-deficient mice.