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Apparent Behavioral Benefits of Tau Overexpression in P301L Tau Transgenic Mice

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Abstract

Transgenic mice expressing human tau containing the P301L tau mutation (JNPL3; tau mice) develop motor neuron loss, paralysis and death between 7 and 12 months. Surprisingly, at 5 and 7 months of age, tau transgenic mice were superior to other genotypes in the rotarod task, and had near perfect scores on the balance beam and coat hanger tests. One tau transgenic mouse was performing at a superior level in the rotarod one day prior to developing paralysis. Cognitive function was also normal in the tau mice evaluated in the radial arm water maze and the Y-maze tasks. We also crossed the tau transgenic mice with Tg2576 amyloid- β protein precursor (A β PP) transgenic mice. Although A β PP mice were deficient in the radial arm maze task, A β PP+tau mice were not impaired, implying a benefit of the tau transgene. Some mice were homozygous for the retinal degeneration mutation (rd/rd) and excluded from the genotype analysis. Only the water maze task discriminated the rd/rd mice from nontransgenic mice. In conclusion, it seems that the modest tau overexpression or the presence of mutant tau in the JNPL3 tau mice may provide some benefit with respect to motor and cognitive performance before the onset of paralysis.

Keywords

tau; amyloid; water maze; rotarod; Y-maze; transgenic mice

INTRODUCTION

The histopathology of Alzheimer's disease reveals two prominent features, amyloid plaques derived from the amyloid- β (A β) peptide and neurofibrillary tangles derived from hyperphosphorylated tau. Mutations in either the amyloid- β protein precursor (A β PP) or in tau lead to neurodegeneration and dementia syndromes (with different clinical presentations;

[11,14]. The tau transgenic line JNPL3 expresses 4 repeat human tau (without N terminal inserts) harboring the FTDP-17 mutation P301L under control of the prion promoter [16]. These mice have little phenotype at birth, but develop a progressive motor abnormality associated with loss of lower motor neurons. These motor abnormalities begin as early as 6.5 months, and have a prevalence of 90% by 10 months, although the phenotype is delayed on certain strain backgrounds. When bred with the Tg2576 A β PP transgenic mouse, there is an increase in the formation of filamentous tau inclusions in forebrain regions [17].

Given that the greatest degeneration in the JNPL3 tau transgenic line is in the spinal cord, we wished to investigate when the earliest motor deficits might be detected. As a first approach, we examined mice at 5 months of age, an age prior to the onset of overt movement abnormalities, using several tasks effective at detecting motor deficits in mice. We tested the same cohort a second time at 7 months, closer to the age of motor disturbance onset and finished with cognitive function testing at 8 months of age. We further compared the tau mice with A β PP+tau mice derived from a cross between Tg2576 and JNPL3 lines to determine if any early motor deficits might be accelerated by the presence of increased A β , as was the case for abnormally phosphorylated tau isoforms in prior work [10,17]

METHODS

Mouse breeding and procedures

Mice were obtained from a cross between Tg2576 mice and JNPL3 mice in the University of South Florida Alzheimer Research Laboratory colony. This cross results in four genotypes, tau only, A β PP only, A β PP+tau and nontransgenic. We started the study with 10 mice in each group except the A β PP mice which had a sample size of 12. All mice were born from the same litters and had a mixed genetic background (contributed by the founder mice) of 54% C57BL/6; 22% DBA/2; 18% SW and 6% SJL. During the study, 1 nontransgenic, 4 A β PP, 4 tau and 1 A β PP+tau mouse died resulting in final sample sizes of 9 nontransgenic, 8 A β PP, 6 tau and 9 A β PP+tau mice at the end of the study. In addition to these mice indicated above, there were 10 mice homozygous for the retinal degeneration (*rd*) mutation. 4 were nontransgenic, 2 were A β PP and 4 were tau genotype. This mutation was contributed by the SW and SJL backgrounds of the parental lines. Although they were tested at the same time as the sighted mice, their data were analyzed separately. All mice were genotyped by PCR for A β PP, tau and *rd* both at weaning and at necropsy. The *rd* mutation genotyping used the primer pairs suggested on the JAX Laboratories web site.

Mice were tested at three ages for behavioral effects of genotype. The first test occurred at 5 months and consisted of the motor test battery of coat hanger test, balance beam and rotarod (see below). A second motor function test was performed at 7 months of age. Finally, cognitive function testing followed the motor function tests at 8 months of age. Brain tissue was then collected for histopathologic analysis of tau tangles (at this age, A β PP mice do not yet have histologically detectable amyloid deposits).

Behavioral testing

Coat hanger task—Strength and coordination were tested in the coat hanger test [15]. In this test, the mouse was suspended by its forepaws from the center of the horizontal portion of a wire coat hanger. The goal was for the mouse to raise its hind legs to the bar, move to the end of the bar and right itself onto the diagonal portion of the coat hanger. Mice are administered 3 trials of up to 60 seconds duration. Each trial was separated by at least 10 minutes. The task was scored by average time on the hanger without dropping (total hang time; presented and Table 1), and an agility score from 1-5 based upon how successful the mice were in reaching the goal (no differences between groups; data not presented).

Balance beam task—As a test of vestibular and general motor function, each animal was carefully placed at the center of a suspended beam and released. The balance time was averaged from 3 successive trials, separated by at least 10 minutes. Maximum trial length was 60 seconds. Because the intention of the balance beam and coat hanger tests was to detect deficits in motor function, the conditions chosen were designed to detect impairments, with most nontransgenic mice performing near maximal performance.

Rotating rod—Mice were placed onto the round portion of a motorized circular rod (Ugo Basile Rota-rod model 7750) which was slowly accelerated starting at 2.5 RPM and accelerate to 34 RPM over 5 minutes with an increase in speed every 30 seconds. Mice were required to walk at the speed of rod rotation to keep from falling. The time until falling was recorded for each mouse. Mice were given three trials each day, separated by at least 10 minutes, for 5 consecutive days. The maximum possible time on the rod was 300 seconds. The average time for each day was recorded. Only very rarely do mice reach the maximum time possible for this task.

Y-maze—Each animal was placed in a walled Y-maze for a single 5 minute trial. The sequence of arm entries and total number of arm choices were recorded. Spontaneous alternation (entering all three arms sequentially without repetition) was expressed as a percentage, as calculated according to the method of {Anisman, 1975 #60}. If an animal made the following sequence of arm selections (1,2,3,2,1,3,1,2), the total alternation opportunities (triads) was 6 (total entries minus 2) and the percentage alternation would be scored as 67% (4 out of 6).

Radial arm water maze—The radial arm water maze has the spatial complexity and ease of performance measurement comparable to the dry radial arm maze combined with the rapid learning normally observed in the Morris water maze without requiring footshock or food deprivation as motivating factors [6]. The radial arm water maze for these studies contained 6 swim paths (arms) radiating from an open central area, with a submerged escape platform located at the end of one of the arms (the goal arm; see [19]). On each trial, the mouse was initially placed in the center of a randomly selected start arm and allowed to swim in the maze for up to 60 seconds to find the escape platform. The platform was located in the same arm on each trial. On day one, mice were given 15 trials alternating between a visible platform (above the water) and a hidden platform (below the water). The next day they were given 15 additional trials with all the trials using a hidden platform. Entry into an incorrect arm (all four limbs within the arm) was scored as an error. If a mouse failed to make an arm entry within 20 seconds, this also was scored as an error. The errors for blocks of 3 consecutive trials were averaged for data analysis.

Mice were organized into cohorts of 4 mice and administered trials in a “spaced” (as opposed to “massed”) manner designed to minimize fatigue. All mice in a cohort received trial 1, then all mice received trial 2, etc. Each cohort was given 3 trials (1 block) sequentially then returned to their home cages while a second cohort was tested. The first cohort was tested then for the second block, alternating with a second cohort until 15 trials (5 blocks) were completed for day 1. The start arm was varied for each trial so that mice rely upon spatial cues to solve the task instead of learning motor rules (i.e., second arm on the right). The goal arm for each successive mouse was different to avoid use of odor cues to locate the goal arm. Group averages of less than 1 error indicate learning of platform location. A detailed description of the general procedure with a few modifications has been published, complete with goal arm assignments and scoring sheets [1].

Histopathology

Mice were euthanatized shortly after completion of radial arm water maze testing by overdose with pentobarbital (200 mg/kg) followed by transcardial perfusion with saline followed by perfusion with neutral buffered paraformaldehyde. Brains were postfixed for 24 hours and cryopreserved in 10%, 20% and then 30% sucrose solutions. Frozen sections at 25 μ m were collected in the horizontal plane through the brain. Every 12th section was stained for abnormally phosphorylated tau using antibody R145d directed against tau phosphoepitope S422 (Biosource International). We chose this antibody originally because Gotz and colleagues [10] found it was the phospho-form of tau that was most sensitive to stimulation by the presence of A β . Earlier work performed in our laboratory with a younger cohort of mice from this breeding had found a correlation between the number of phospho-tau cells per slide and performance on spatial navigation and visible platform tasks [3]. We used this same antibody again because we were attempting to confirm this observation. We counted all positively stained profiles in the hippocampus and cortex of these mice (this analysis was performed by the same individual as in [3]). Because the A β PP mice were collected at an age before deposits appeared, we did not stain for amyloid load in these mice.

Statistics

Genotype effects were analyzed by ANOVA followed by means testing with Fisher's LSD analysis, using the statistical analysis program Statview (SAS, Chicago IL). For the rotarod and radial arm water maze tasks, days or blocks were included in a 2 way ANOVA as a repeated measure. $P < 0.05$ was selected as the criterion for statistical significance.

RESULTS

Motor function testing

Mice were given a motor function battery at two ages, in anticipation of detecting early signs of lower motor neuron dysfunction in the tau transgenic mice. The first age was 5 months, an age well in advance of the onset of hindlimb paralysis in prior work. A second age of 7 months was chosen as the earliest time when mice might exhibit paralysis. Mice were subjected to a short motor test battery consisting of a coat hanger test on one day, a balance beam task on a second day, and 5 consecutive days of rotarod testing.

All mice did well on the coat hanger test at 5 months. The mean hang times ranged from 46 to 54 (out of 60) seconds, and no differences were detected between the four genotypes (Table 1). A similar outcome was obtained for the balance beam task in 5 month old mice (Table 1). The rotarod testing revealed that all groups of mice improved performance over days, and mouse genotype had an effect on the time spent on the rod (Figure 1; repeated measures 2 way ANOVA; significant effect of genotype, $P < 0.05$, and a significant effect of days, $P < 0.001$). Somewhat unexpectedly, the best performing mice were those expressing the tau transgene. When averaged across all 5 days, the tau mice performed significantly better than the nontransgenic mice ($P < 0.05$) and the A β PP mice ($P < 0.01$). The A β PP, nontransgenic and A β PP+tau groups did not differ from each other.

At 7 months of age, the coat hanger test again indicated comparable performance among the 4 genotypes (Table 1). In the balance beam test, the A β PP+tau mice were inferior to the other three groups (Table 1). At 7 months, rotarod testing again indicated a significant effect of days of training ($P < 0.001$) and of genotype ($P < 0.005$; Figure 1). Analysis of the average of all 5 days indicated that the tau mice performed better than either the nontransgenic mice ($P < 0.05$) or the A β PP+tau mice ($P < 0.001$). In addition, the A β PP+tau mice performed significantly worse on the rotarod than the A β PP only mice ($P < 0.05$), but not nontransgenic mice (Figure 1).

Given the fact that transgenic tau expression in this mouse line ultimately caused paralysis, it seemed surprising that these mice exhibited superior performance on the rotarod task. We did note that one mouse became paralyzed during the testing procedure which led us to examine the performance of individual mice over days. In Figure 2, individual mouse values are plotted in comparison to the mean and SEM for nontransgenic animals (solid line). Two mice with typical individual performances for tau transgenic mice are mouse 4 (solid circles) and mouse 34 (open circles). Their times were above the nontransgenic mean value on the first day, and progressively improved over the 5 days of the trial. Conversely, mouse #41 (open diamonds) started the first 3 days of rotarod testing considerably above the average for nontransgenic mice. Remarkably, on the fourth day the performance of mouse 41 had deteriorated and by the end of the session it was noticed that hind limb paralysis had set in. This mouse was unable to perform the rotarod task on day 5 and was euthanatized shortly thereafter. Another tau animal, mouse 40 (solid triangles), showed a progressive deterioration of performance over 5 days (an unusual response), but did not develop hindlimb paralysis before tissue was collected after the cognitive function tests. One potential explanation for such rapid changes in behavioral performance might be explained by increased neuronal activity, which might both improve motor performance, but also increase vulnerability to excitotoxic forms of damage.

Cognitive function testing

Mice were evaluated in the Y maze and in a 2 day version of the radial arm water maze shortly after completing the motor function testing at 7-8 months of age. No differences were detected in Y-maze alternation performance among the 4 genotype groups (Figure 3A). All groups had mean alternation percentages around 60%, typical for control mice in our prior studies [4,9,12,13,19,29]. However, the mice overexpressing the A β PP transgene exhibited an increased number of arm entries (Figure 3B), an observation consistent with our prior work (cited above).

The 2 day radial arm water maze was used to assess spatial navigation learning and memory. In this task, nontransgenic mice showed continuous improvement in the task, achieving the less than 1 error criterion for learning by block 7 (Figure 4). A β PP mice were deficient in learning the platform location in this task. Repeated measures ANOVA indicated a significant effect of blocks ($P < 0.001$) indicating that overall learning occurred during the 2 days and a significant effect of genotype ($P < 0.005$). Means analysis at individual data points indicated that A β PP mice performed significantly worse than all three other groups during blocks 6, 8 and 10 ($P < 0.01$ or greater for all comparisons). The important observation is that the A β PP+tau mice performed significantly better than the A β PP only transgenic mice, suggesting that the two transgenes not only failed to synergize, but the tau transgene rescued the effect of the A β PP transgene on memory performance.

We also counted the number of neurons in tau and A β PP+tau transgenic mice that were labeled with antisera against phospho-ser 422 of human tau. Not all mice carrying a tau transgene exhibited staining for this abnormally phosphorylated form of tau in forebrain, and the numbers of such neurons in cortex or hippocampus were small in number, limiting the analysis to manual counts. We found a trend towards higher numbers of tau positive neurons per section in hippocampus of A β PP+tau mice (50 ± 27) versus tau only mice (3.1 ± 1.6) but this effect was not significant ($P < 0.09$). Still this trend is consistent with earlier work combining these lines where the strongest effects were observed in older mice than those examined here [17]. In cortex, a similar trend was present (8.9 ± 4.9 versus 3.8 ± 1.7 ; A β PP+tau versus tau). None of the A β PP only or nontransgenic mice had cells positive for this form of phospho-tau. We then attempted to correlate the number of phospho-tau positive neurons with cognitive performance on the radial arm water maze task. We correlated cognition and pathology in tau mice and A β PP+tau mice separately and also correlated

behavior and pathology with the tau plus A β PP+tau mice combined. We compared the phospho-tau neuron numbers with several indices of learning task performance. None of these showed a correlation above 0.15, indicating these phenomena were unlinked.

Effects of homozygosity for the retinal degeneration mutation

Mice on this mixed genetic background, integrating the P301L tau transgenic mouse background and the Tg2576 A β PP transgenic mouse background, resulted in an unexpectedly large number of mice that were homozygous for the *rd*, retinal degeneration mutation. Although initially included during the behavioral testing of the mice, these blind mice were excluded from the data analyses presented above. We noticed, however, that these mice were largely indistinguishable from sighted mice on the balance beam task, the coat hanger task (Table 1), and the Y maze task (Figure 3). Even on the rotarod, they performed as well as nontransgenic mice (Figure 5A). The only task capable of distinguishing these blind mice from sighted mice was the radial arm water maze task. In Figure 5B, it is clear that these mice do not improve over blocks on this task, yet the number of errors overall is not substantially different than sighted mice that are unaware of the platform location (3-4 errors is typically under these circumstances).

DISCUSSION

The key observation in this study is that P301L tau transgenic animals, destined to develop hind limb motor paralysis secondary to straight or wavy tau filament formation [18] and death of lower motor neurons [16], do not exhibit antecedent deficits in motor behavior. Neither at 5 nor 7 months of age do these mice exhibit impairments on several tasks often used to detect motor abnormalities in transgenic mice. Remarkably, the performance of these mice on the rotarod task is actually superior to that of the other genotypes. However, the benefits of the tau transgene did not extend to the A β PP+tau transgenic mice, as their performance was not significantly different from the nontransgenic mice.

These results were certainly unexpected, given the anticipation that tau transgenic mice of the JNPL3 line invariably develop hindlimb paralysis. This is associated with oligodendrocytic apoptosis and axonal degeneration in the spinal cord, with 50% loss of lower motor neurons in mice exhibiting motor abnormalities [16,18,30]. Thus, we designed these studies to detect early deficits in motor performance. It is uncertain if the enhancements observed with the rotarod task would generalize to other motor abilities using tasks sensitive to improvements in performance. A recent study indicated that JNPL3 tau mice surviving to 12 months and exhibiting motor abnormalities are impaired in the rotarod task [21], but earlier testing was not reported. In the THY-tau22 mouse model, Schindowski and colleagues [27] report that the transgenic mice stayed on the rotarod 30% longer on average than nontransgenic mice, although the effect did not reach statistical significance. Certainly, the balance beam and coat hanger task, often used successfully to detect motor deficits, failed to indicate any impairments in the tau animals. However, the task conditions were designed to detect deficits and most mice perform near the ceiling. Thus, they are relatively insensitive to improvements in motor performance. The rotarod is probably the only task in this series that has room for mice to improve, and, thus, is uniquely sensitive to detect enhancements in motor capability.

By inference, the cognitive function of the A β PP mice was improved by the presence of the tau transgene, as the A β PP+tau mice performed better than A β PP only mice in the last blocks of the radial arm water maze task. There was no difference in performance between tau mice and nontransgenic littermates. It should be noted that the apparent learning deficit in the A β PP+tau mice is not an artifact of failing to make arm choices, as mice failing to enter arms would receive a score of 3 (see Methods). Thus, on balance, the tau transgene

effect on cognition tended towards being beneficial, rather than detrimental. These data are very similar to those obtained earlier with mice derived from the same breeding pairs as the present study. Arendash et al. [3] found impaired radial arm water maze function in A β PP mice, but no effect of the tau transgene compared to nontransgenic littermates. Unfortunately, this cohort lacked a sufficient number of tau+A β PP mice for a separate analysis, so they were grouped with the tau only animals for data analysis. In spite of the absence of a main effect of tau genotype on spatial navigation behavior, Arendash and colleagues [3] reported three significant correlations between a behavioral measure and the numbers of phospho-tau positive cells in one of the 5 brain regions evaluated (data collected by our research group). Our laboratory had concerns regarding the reliability of these correlations due to the extensive number of behavioral tests used (32) and the separate correlation with each of 5 brain regions (150 possible correlations). It seemed likely to us that some correlations would be positive by chance alone. Our study, using a larger dataset, restricted number of behavioral test values, and focused analysis on cortical and hippocampal cell numbers, failed to confirm these correlations. Although minor differences in the behavioral procedure and analysis of a different cohort of mice (albeit litters from the same transgenic parents) might explain the differences between these studies, we suggest that the initial observation resulted by chance due to the large number of correlations performed without error correction procedures.

At face value, these data suggest that modest overexpression of tau, or the presence of mutant tau, can be beneficial. However, it is important to recognize that in the absence of data on levels of tau expression within individual populations of neurons in JNPL3 mice, especially lower motor neurons, it is not possible to determine what levels of expression correspond to this apparent benefit. In a different P301L tau transgenic line, Boekhoorn et al. [5] reported that the tau mice at young ages (3 months) had improved object recognition performance and object recognition memory compared to nontransgenic mice. In the young THY-tau22 model, there is also evidence for increased neurogenesis [28]. Intriguing support for this argument also derives from work examining a different human tau transgenic line. These mice express a human PAC, termed 8c mice [7], but develop very little tau pathology. It is only when bred onto the tau knockout background that these mice develop neurofibrillary tangles and neuron loss [2], implying the endogenous mouse tau was somehow protective against the neuropathology. However, the opposite conclusion was reached in a well conducted study breeding A β PP mice onto a tau null background [24]. The tau null background had no impact on amyloid deposition in these mice, but rescued the memory deficits exhibited by these mice when bred on a tau sufficient background.

Certainly extensive overexpression of tau can have deleterious consequences. SantaCruz and coworkers [26] demonstrated that a mouse expressing human P301L tau under control of the tetracycline response element (rTg4510) developed early and severe neuropathology, neuron loss and memory disruption. The memory loss was reversed when the transgene was suppressed with doxycycline, even though the neurofibrillary tangles continued to develop. Intriguingly, the doxycycline suppression reduced the expression of the tau transgene from 15 fold overexpression to 2.5 fold overexpression relative to endogenous mouse tau. In contrast, the average overexpression of the tau transgene in the JNPL3 is estimated as 2 fold endogenous murine expression. However, tau expression levels within spinal cord neurons that degenerate selectively are not known. Several of the tau mouse lines also exhibit memory deficits, but in general these do not impact all cognitive domains, nor are the effects as profound as those seen in A β PP transgenic mice unless severe neuron loss is present [20,23,25,27].

One unexpected outcome from the breeding between the Tg2576 A β PP mice and the JNPL3 tau mice was a large number of mice homozygous for the retinal degeneration mutation.

This was most likely introduced through the SJL mice on the Tg2576 line and the SW background of the JNPL3 mice. Perhaps most remarkable is that the behavior of these mice was indiscernible from the behavior of mice with intact vision on most tasks, indicating that mice compensate for visual losses quite effectively. In fact, in prior work these mice even “passed” the visual cliff task intended to detect visual impairments in a neurological test battery [8]. Only the spatial navigation task revealed the considerable visual deficit. Importantly, the mice typically made 3–4 errors, a number consistent with mice skilled in the task, but without knowledge of the location of the platform. These mice appear to use an efficient search strategy, establishing a mental map of the maze and not repeating arms. It is simply that they cannot use the extramaze cues to orient their starting position within the maze, and thus are unable to determine the platform location when started in different arms each trial.

These rd/rd mice perform similarly to several inbred mouse lines previously demonstrated incapable of learning the Morris water maze, a spatial navigation task relying upon the same visuospatial skills used in the radial arm water maze [22]. The poor learning strains included FVB, C3H, SJL, A, BUB/BnJ and Balb/c. Of these lines, FVB, C3H, SJL, and BUB/BnJ are known to be homozygous for the rd mutation (see www.jax.org/jaxmice). In studies by Owen and colleagues of F1 hybrids [22], all hybrids performed well, even when one parent was from a line carrying the rd mutation, indicating heterozygosity at this allele lacks a major behavioral phenotype. Importantly, Owen et al. [22] predicted there was a sensory deficit in these poorly performing mouse lines, as these mice also did poorly on the visible platform test. This highlights the use of the visible platform as a task to detect mice lacking the sensory apparatus necessary to learn spatial navigation tasks.

In conclusion, these behavioral results fail to confirm the initial hypothesis that JNPL3 mice would have antecedent motor impairments at early ages as phospho-tau species accumulate in their lower motor neurons. Surprisingly, we find the opposite effect, of enhanced running time on the rotarod task in tau transgenic mice. Coupled with other observations reported recently, these data suggest that modest levels of tau overexpression may provide some benefits to neurons, that are only overcome when levels of specific toxic tau species reach critical concentrations, and cause neurodegeneration. The basis for this benefit is unclear, however, the well established role of tau in stabilizing microtubules may provide a potential explanation in that ultra-stable microtubules may enable improved or more robust axonal function. This effect would likely be prominent in motor neurons due to their long axons which may in turn explain the impact on motor function in the rotarod test.

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References

1. Alamed J, Wilcock DM, Diamond DM, Gordon MN, Morgan D. Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice. *Nat Protoc* 2006;1:1671–1679. [PubMed: 17487150]
2. Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *Journal of Neuroscience* 2005;25:5446–5454. [PubMed: 15930395]
3. Arendash GW, Lewis J, Leighty RE, McGowan E, Cracchiolo JR, Hutton M, Garcia MF. Multi-metric behavioral comparison of APPsw and P301L models for Alzheimer’s disease: linkage of poorer cognitive performance to tau pathology in forebrain. *Brain Res* 2004;1012:29–41. [PubMed: 15158158]

4. Austin L, Arendash GW, Gordon MN, Diamond DM, DiCarlo G, Dickey C, Ugen K, Morgan D. Short-term beta-amyloid vaccinations do not improve cognitive performance in cognitively impaired APP + PS1 mice. *Behav Neurosci* 2003;117:478–484. [PubMed: 12802876]
5. Boekhoorn K, Terwel D, Biemans B, Borghgraef P, Wiegert O, Ramakers GJ, de Vos K, Krugers H, Tomiyama T, Mori H, Joels M, van Leuven F, Lucassen PJ. Improved long-term potentiation and memory in young tau-P301L transgenic mice before onset of hyperphosphorylation and tauopathy. *J Neurosci* 2006;26:3514–3523. [PubMed: 16571759]
6. Diamond DM, Park CR, Heman KL, Rose GM. Exposing rats to a predator impairs spatial working memory in the radial arm water maze. *Hippocampus* 1999;9:542–551. [PubMed: 10560925]
7. Duff K, Knight H, Refolo LM, Sanders S, Yu X, Picciano M, Malester B, Hutton M, Adamson J, Goedert M, Burki K, Davies P. Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. *Neurobiol Dis* 2000;7:87–98. [PubMed: 10783293]
8. Garcia MF, Gordon MN, Hutton M, Lewis J, McGowan E, Dickey CA, Morgan D, Arendash GW. The retinal degeneration (rd) gene seriously impairs spatial cognitive performance in normal and Alzheimer's transgenic mice. *NeuroReport* 2004;15:73–77. [PubMed: 15106834]
9. Gordon MN, King DL, Diamond DM, Jantzen PT, Boyett KL, Hope CE, Hatcher JM, DiCarlo G, Gottschall P, Morgan D, Arendash GW. Correlation between cognitive deficits and A β deposits in transgenic APP+PS1 mice. *Neurobiology of Aging* 2001;22:377–385. [PubMed: 11378242]
10. Gotz J, Chen F, Van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by A β 42 fibrils. *Science* 2001;293:1491–1495. [PubMed: 11520988]
11. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;297:353–356. [PubMed: 12130773]
12. Holcomb LA, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 1998;4:97–100. [PubMed: 9427614]
13. Holcomb LA, Gordon MN, Jantzen P, Hsiao K, Duff K, Morgan D. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: Lack of association with amyloid deposits. *Behav Genet* 1999;29:177–185.
14. Hutton M. Missense and splice site mutations in tau associated with FTDP-17: multiple pathogenic mechanisms. *Neurology* 2001;56:S21–S25. [PubMed: 11402146]
15. Lalonde R, Thifault S. Absence of an association between motor coordination and spatial orientation in lurcher mutant mice. *Behav Genet* 1994;24:497–501. [PubMed: 7872930]
16. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Paul MM, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet* 2000;25:402–405. [PubMed: 10932182]
17. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 2001;293:1487–1491. [PubMed: 11520987]
18. Lin WL, Lewis J, Yen SH, Hutton M, Dickson DW. Ultrastructural neuronal pathology in transgenic mice expressing mutant (P301L) human tau. *J Neurocytol* 2003;32:1091–1105. [PubMed: 15044841]
19. 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]
20. Murakami T, Paitel E, Kawarabayashi T, Ikeda M, Chishti MA, Janus C, Matsubara E, Sasaki A, Kawarai T, Phinney AL, Harigaya Y, Horne P, Egashira N, Mishima K, Hanna A, Yang J, Iwasaki K, Takahashi M, Fujiwara M, Ishiguro K, Bergeron C, Carlson GA, Abe K, Westaway D, St George-Hyslop P, Shoji M. Cortical neuronal and glial pathology in TgTauP301L transgenic mice:

- neuronal degeneration, memory disturbance, and phenotypic variation. *Am J Pathol* 2006;169:1365–1375. [PubMed: 17003492]
21. Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, Gaynor K, Wang L, LaFrancois J, Feinstein B, Burns M, Krishnamurthy P, Wen Y, Bhat R, Lewis J, Dickson D, Duff K. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci USA* 2005;102:6990–6995. [PubMed: 15867159]
 22. Owen EH, Logue SF, Rasmussen DL, Wehner JM. Assessment of learning by the morris water task and fear conditioning in inbred mouse strains and F1 hybrids: Implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neurosci* 1997;80(4):1087–1099.
 23. Pennanen L, Wolfer DP, Nitsch RM, Gotz J. Impaired spatial reference memory and increased exploratory behavior in P301L tau transgenic mice. *Genes Brain Behav* 2006;5:369–379. [PubMed: 16879631]
 24. 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]
 25. Rosenmann H, Grigoriadis N, Eldar-Levy H, Avital A, Rozenstein L, Touloumi O, Behar L, Ben-Hur T, Avraham Y, Berry E, Segal M, Ginzburg I, Abramsky O. A novel transgenic mouse expressing double mutant tau driven by its natural promoter exhibits tauopathy characteristics. *Exp Neurol*. 2008
 26. Santacruz K, Lewis J, Spire T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH. Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 2005;309:476–481. [PubMed: 16020737]
 27. Schindowski K, Bretteville A, Leroy K, Begard S, Brion JP, Hamdane M, Buee L. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am J Pathol* 2006;169:599–616. [PubMed: 16877359]
 28. Schindowski K, Belarbi K, Bretteville A, Ando K, Buee L. Neurogenesis and cell cycle-reactivated neuronal death during pathogenic tau aggregation. *Genes Brain Behav* 2008;7(Suppl 1):92–100. [PubMed: 18184373]
 29. Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, Morgan D. Passive immunotherapy against Abeta 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: 15588287]
 30. Zehr C, Lewis J, McGowan E, Crook J, Lin WL, Godwin K, Knight J, Dickson DW, Hutton M. Apoptosis in oligodendrocytes is associated with axonal degeneration in P301L tau mice. *Neurobiol Dis* 2004;15:553–562. [PubMed: 15056463]

Figure 1.

Enhanced rotarod performance in P301L tau transgenic mice. Four genotypes of mice, tau (T; solid triangles $n = 9$), A β PP+tau (A+T; open diamonds; $n = 9$), nontransgenic mice (solid squares; solid line; $n = 9$) and A β PP mice (A; solid circles; $n = 11$) were tested in the rotarod for 3 trials each day for 5 consecutive days. Panel A is testing performed when mice were 5 months of age and panel B represents testing of the same mice at 7 months of age. Tau mice differed from nontransgenic mice at both ages (see text for detailed statistical results).

Figure 2.

Patterns of individual mouse performances in the rotarod testing. The mean $\pm$ standard error for nontransgenic (N) mice is represented by the solid line. Individual values for tau mice numbers 4 (solid circles), 34 (open squares), 41 (open diamonds) and 40 (solid triangles) are shown. Mouse # 41 developed hindlimb paralysis during the testing procedure.

Figure 3.

Increased activity in A β PP transgenic mice. Mice were tested in the Y maze for 5 minutes and number of arm entries and % alternation recorded. As seen in a number of prior studies, mice with the A β PP transgene made a larger number of arm entries compared to nontransgenic animals. All mice exhibited normal alternation performance

Figure 4. Radial arm water maze performance deficits in A β PP transgenic mice are reversed by the presence of the tau transgene. Mice were run in the radial arm water maze to measure spatial navigation learning and performance. A β PP mice (solid circles) performed significantly worse than nontransgenic (solid squares), tau transgenic (solid triangles) or A β PP+tau (open diamonds) mice in several blocks on day 2 of training (see text for detailed statistical analyses).

Figure 5. Performance of blind (rd/rd) mice on rotarod and radial arm water maze tasks. Mice homozygous for the retinal degeneration mutation rd/rd performed comparably to the nontransgenic mice on the rotarod task (panel A; 7 month measurement). In the behavioral tests performed here, the only deficiency detected in the rd/rd mice was in the radial arm water maze (panel B) where the mice failed to improve significantly over the 2 days of testing.

Table 1**Motor Performance Testing**

Motor Test	NonTransgenic	AβPP	Tau	AβPP+Tau	rd/rd
5 month Coat Hanger	54 $\pm$ 4	46 $\pm$ 4	52 $\pm$ 4	49 $\pm$ 4	47 $\pm$ 3
5 month Balance Beam	47 $\pm$ 6	44 $\pm$ 6	50 $\pm$ 5	36 $\pm$ 7	40 $\pm$ 7
7 month Coat Hanger	47 $\pm$ 3	44 $\pm$ 4	45 $\pm$ 5	39 $\pm$ 6	45 $\pm$ 2
7 month Balance Beam	38 $\pm$ 6	44 $\pm$ 6	40 $\pm$ 7	18 $\pm$ 4 *	53 $\pm$ 3

* Significantly lower than A β PP, tau and nontransgenic mice, $P < 0.01$, 0.02 and 0.05, respectively