Published in final edited form as:

Behav Brain Res. 2012 July 15; 233(1): 141–148. doi:10.1016/j.bbr.2012.04.034.

Adult-onset focal expression of mutated human tau in the hippocampus impairs spatial working memory of rats

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Abstract

Tauopathy in the hippocampus is one of the earliest cardinal features of Alzheimer's disease (AD), a condition characterized by progressive memory impairments. In fact, density of tau neurofibrillary tangles (NFTs) in the hippocampus strongly correlates with severity of cognitive impairments in AD. In the present study, we employed a somatic cell gene transfer technique to create a rodent model of tauopathy by injecting a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the hippocampus of adult rats. The P301L mutation is causal for frontotemporal dementia with parkinsonism-17 (FTDP-17), but it has been used for studying memory effects characteristic of AD in transgenic mice. To ascertain if P301L-induced mnemonic deficits are persistent, animals were tested for 6 months. It was hypothesized that adult-onset, spatially restricted tau expression in the hippocampus would produce progressive spatial working memory deficits on a learned alternation task. Rats injected with the tau vector exhibited persistent impairments on the hippocampal-dependent task beginning at about 6 weeks post-transduction compared to rats injected with a green fluorescent protein vector. Histological analysis of brains for expression of human tau revealed hyperphosphorylated human tau and NFTs in the hippocampus in experimental animals only. Thus, adult-onset, vector-induced tauopathy spatially restricted to the hippocampus progressively impaired spatial working memory in rats. We conclude that the model faithfully reproduces histological and behavioral findings characteristic of dementing tauopathies. The rapid onset of sustained memory impairment establishes a preclinical model particularly suited to the development of potential tauopathy therapeutics.

Keywords

AAV; Hippocampus; Learning; Memory; P301L; Tauopathy

1. Introduction

Tauopathies are a class of neurodegenerative diseases characterized by abnormal phosphorylation and aggregation of the microtubule-associated protein tau. Alzheimer's disease (AD), frontotemporal dementia with parkinsonism linked to tau mutations on

chromosome 17 (FTDP-17), progressive nuclear palsy (PSP), and Pick's disease share these features of tau pathology [1]. Of these tauopathies, AD is the most common, affecting an estimated 30% of those over age 80 [2]. Although the brains of AD patients typically exhibit mixed tau neurofibrillary tangle (NFT) and amyloid plaque pathology, beta-amyloid has received extensive attention as a primary causative agent of neurodegeneration and AD-specific memory impairments [2–8]. It is only recently that the focus has begun to shift to tau as a central causative agent of AD pathology [9–13]. In both inherited and sporadic human tauopathies, tau is abnormally hyperphosphorylated on serine, threonine, and tyrosine residues [14–20]. Functional inactivation of hyperphosphorylated, aggregation-prone tau, the generation of abnormally truncated tau, and accumulation of intra- and extracellular tau participate in a pathogenic cascade resulting in the formation of NFTs, pretangles, neuropil threads, and compromised microtubule-dependent intracellular transport. In AD, density of NFTs in the hippocampus strongly correlates with cognitive impairments and neuronal loss [21,22]. Although distinct diseases, AD and FTDP-17 caused by tau mutations such as P301L share NFT pathology. Experimental models based on FTDP-17 tau have thus provided relevant insight into tauopathy common to a class of progressive dementing neurodegenerative diseases [23–25].

Most work on tauopathies in animal models to date has been on transgenic models of tauopathy. This technology is most advanced in mice, but transgenic rat models are also available [18]. Conditional transgenic mouse models offer experimental control of onset and region of transgene expression that may better approximate diseases that develop in adults than constitutively expressing mouse models can. For instance, the 'tet-off' gene expression system can be used to switch off expression of the mutant tau gene starting at a specific age and restricting it to a specific brain region, which avoids the possible confounds of behavioral abnormalities being due to transgene expression in a different brain region or the transgene expression having interfered with a process during development [18,26–28]. Experimental gene transfer of disease-mutant human tau genes is an alternative approach to generate animal models of tauopathy. It offers an even greater experimental control of onset, location, and degree of expression of the transgene than conditional transgenic animal models do. This can more closely approximate natural disease, such as the relatively restricted localization of tauopathy in temporal and frontal regions in FTDP-17, and to eliminate confounds related to expression of disease genes in brain regions not affected in the natural disease, such as cerebellum or relatively spared cortical subregions [29–31]. The precise localization and timing of disease gene expression can be used to identify brain regions where damage underlies specific functional phenotypes. For example, tau expression restricted to the entorhinal cortex is sufficient to impair performance on a hippocampal-dependent learned alternation task [12].

Since the discovery of the AAV9 serotype in 2004 by Gao et al. [32], this variant has proven to be efficient for gene delivery to the CNS [33,34]. The recombinant AAV9 serotype has been successfully used to induce P301L tau expression in rats and was associated with profound neurodegeneration [35–37]. Of note is the fact that the time course of mutant human tau expression with AAV9 is rapid, showing faster onset and stronger expression of P301L tau than AAV2 and AAV8 [36]. AAV_{P301L} tau is expressed by 1 week, neurotransmitter loss can be observed by 2 weeks, and behavioral effects are evident by 3–4 weeks after transduction [37].

In order to recapitulate features of an adult-onset progressive human tauopathy with limited brain distribution, we injected a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the hippocampus of adult rats. The P301L mutation causes human tau to polymerize and form straight filaments, hyperphosphorylated insoluble tau, and NFTs [24,38–41]. We targeted the hippocampus because it plays a key role in learning

and memory [42–46], because NFTs appear early there in tauopathies such as AD and FTDP-17, and because symptom severity in AD has been linked to density of tau-composed NFTs in this location [47]. Finally, hippocampal shrinkage and decline in synaptic density are also evident in AD and are likely contributors to the mnemonic dysfunction associated with the disease [48,49]. The objective of the study was to determine whether adult-onset, focally expressed tauopathy in the hippocampus produces persistent mnemonic deficits on a spatial working memory task. Our study sought to assess the ability of tau_{p301L} transduction to produce mnemonic deficits over six months, a time course longer than in previous studies, to examine whether the rapid disruptive effects of tau_{p301L} on memory progress or even persist over time. We predicted that we would see clear histological evidence of tauopathy in the hippocampus after six months, along with persistent impairment on the spatial memory task, as would be expected of a relevant animal model of tauopathy if tau pathology in the hippocampus contributes to mnemonic impairment [18]. Such a result would indicate a valid use of rAAV9 tau_{p301L} transduction as a tool in therapeutic drug discovery for adult-onset tauopathies such as AD.

2. Material and methods

2.1. Subjects

Twenty male Sprague-Dawley rats weighing (325–350 g; approximately 90 days of age at the start of the behavioral testing) were purchased from Hilltop Animal Labs Inc. (Scottsdale, PA). Animals were housed individually in cages and maintained on a 12 h light-dark cycle (lights on at 7:00 am). Animals were food deprived to about 80% of their initial body weight at the beginning of the investigation; they were subsequently allowed to gain up to 5 g each week for the remainder of the testing period. The rats had *ad libitum* access to water. All procedures described here were approved by the Davidson College Institutional Animal Care and Use Committee and were conducted in accordance with the guidelines of the Animal Welfare Act and the National Institutes of Health.

2.2. Apparatus

A gray semi-automated Y-maze was used for all behavioral testing. An approach alley (13 cm high × 13 cm wide × 40 cm long) was separated from two goal arms (13 cm high × 13 cm wide × 47 cm long) by a guillotine door. Among the advantages of this apparatus over other behavioral tasks are its high sensitivity at detecting memory impairments [50], its ability to tap into spatial working hippocampal memory, and its utility over other methods (such as spontaneous alternation) as a measure of retention [51].

2.3. Experimental design and behavioral testing

The rats were randomly assigned to two treatment conditions: (1) bilateral hippocampal injection of rAAV9-tau_{p301L} (tau, $n = 10$); or (2) bilateral hippocampal injections of rAAV9-GFP (GFP control, $n = 10$). The behavioral testing procedures were similar to those reported earlier [12,52]. Briefly stated, before surgery the rats were trained for 1 week to traverse a maze for food reward (two 45 mg Noyes pellets) according to a random series of forced left and right turns (*i.e.*, the Gellerman sequence) as employed in previous studies [12,52]. Ten days following surgery, the rats were trained to alternate in the Y-maze for two 45 mg Noyes pellets for 11 trials per day (for a total of 10 alternations per training session with 40 s inter-trial intervals). After receiving a reward for the first trial of a session, the rats were reinforced only for correctly alternating their entry into a goal arm on subsequent trials. Rats were tested 5 days per week for 12 consecutive weeks, then for one week per month for three months. The experimenters were blind to the rats' treatment conditions. Errors were defined as repeated entry into a previously rewarded goal arm, perseverative

errors were defined as two consecutive mistakes, and criterion for acquisition was operationally defined as committing two or fewer errors for 3 consecutive days [12,53].

2.4. AAV9 vector preparation

We made recombinant AAV9 vectors for either human tau or control GFP transgenes using previously described methods [36]. The form of the tau transgene contained the P301L mutation associated with inherited frontotemporal dementia [54,55], and four microtubule-binding domains (including exons 2/3/10). The expression cassette was flanked with AAV2 terminal repeats. The hybrid cytomegalovirus/chicken β -actin promoter and the woodchuck hepatitis post-transcriptional regulatory element were used to drive and enhance expression [56]. Human embryonic kidney 293-T cells were co-transfected with either the tau or the GFP transgene plasmid along with the packaging plasmid needed to make AAV9 [32,34]. The cell lysate was applied to a discontinuous gradient of iodixanol (OptiPrep, Greiner Bio-One, Longwood, FL) and centrifuged. The AAV fraction was then removed, diluted 2-fold with lactated Ringer's solution (Baxter, Deerfield, IL), and then washed and concentrated with Millipore (Billerica, MA) Biomax 100 Ultrafree-15 units. The final stocks were sterilized with Millipore Millex-GV syringe filters and collected into low adhesion tubes (USA Scientific, Ocala, FL). Vectors were aliquoted and stored frozen. Encapsidated genome copies were titered by dot-blot. Equal vector dose comparisons were made by normalizing titers with the diluent, lactated Ringer's solution.

2.5. Surgical procedures

Following behavioral training on the Gellerman sequence, the rats sustained surgery under aseptic conditions. After 12 h of food and water deprivation, the rats were anesthetized using 4% isoflurane gas anesthesia (flow rate of 200–400 μ L/min) that was later adjusted to 2.5%. A craniotomy overlying the hippocampus was performed and injections of either the rAAV9-GFP or rAAV9-tau_{P301L} vectors were made into the following stereotactically defined sets: incisor bar set at +5.0 mm; AP: 2.0, ML: $\pm$ 1.5, DV: –3.0; and AP: –4.2, ML: $\pm$ 4.5, DV: –5.0. A 22° beveled tip 10 μ L 26 gauge Stoelting Hamilton syringe (51096S) was loaded with vector and slowly inserted to the desired depth over the course of 2 min in one hemisphere. The needle was allowed to sit in the brain for an additional 2 min prior to injection of the viral vector. Subsequently, viral vector was injected with a Stoelting Nano-Injector (Stoelting, Co., Wood Dale, Illinois) for microinjection at a rate of 0.15 μ L/min of viral vector over the course of 20 min for a total of 3.0 μ L in each of the four injection sites. The two sites per side strategy was employed to target both rostro-dorsal and caudo-ventral hippocampus. Tau vector contained 1.1×10^9 vector genomes per μ L. Thus, the injections totaling 12 μ L per rat amounted to a dose of 1.32×10^{10} vector genomes per tau rat. GFP vector originally contained 7.2×10^9 vector genomes per μ L and was then diluted to 1.1×10^9 vector genomes per μ L so that the injections totaling 12 μ L per rat amounted to a dose of 1.32×10^{10} vector genomes per control rat. After 2 min, needles were removed over the course of 2 min and incisions closed by suture. Postoperative behavioral testing began within 7–10 days following surgery according to the methods described above.

2.6. Histological procedures

After completion of the behavioral testing, rats were anesthetized deeply with sodium pentobarbital (Nembutal, 100 mg/kg ip) and transcardially perfused with 100 ml 0.1 M sodium chloride chased by 400 ml 4% formaldehyde in 0.1 M phosphate buffered saline pH 7.4 (PBS). After 2 h at 4 °C the brains were extracted into 30% sucrose PBS for cryoprotection and stored refrigerated. The cerebellum was removed and the frontal pole separated by a coronal cut between the septum and hippocampus. The block containing hippocampus was sectioned at –18 °C in the horizontal plane using a sliding microtome fitted with a thermoelectric freezing stage. Sections (50 μ m) were placed in individual wells

of 24-well polypropylene plates (Phenix Research Products, Inc., Candler, NC) containing 400 μ l PBS. Sections for immunolabeling were subjected to suppression of endoperoxidative activity by incubation in a 400 μ l/well solution of 3% hydrogen peroxide in PBS for 5 min, followed by 2×5 min washes in 500 μ l PBS/well. All incubations were conducted on a rotating shaker at ambient temperature (*ca.* 21 °C). Non-specific binding was blocked by incubation in 2% goat serum in PBS. Primary antibodies (GFP, Molecular Probes A11122, 1:10,000; T14, Zymed 13-1400, 1:1000; AT8, Pierce Endogen, 1:500; NFT, Chemicon AB1518, 1:200; GFAP, Sigma G3893, 1:400; ED-1, Serotec, 1:500; OX6, Serotec, 1:1000; 8-hydroxy-deoxyguanosine, Sigma, 1:1000), diluted in PBS containing 0.1% Triton X-100 detergent plus 0.02% antimicrobial sodium azide, were incubated overnight at 200 μ l/well. Following 2×15 min washes of 500 μ l PBS/well, sections were incubated overnight in biotinylated goat anti-rabbit (Dako E0432, 1:1000) or anti-mouse (Sigma B0529, 1:10,000) secondary antibodies in PBS. After 2 more PBS washes, sections were incubated overnight in extravidin-peroxidase (Sigma E2886, 1:1000), washed twice with a 500 μ l/well solution of 0.1 M sodium acetate, and reacted for 5 min with a chromogen solution of 0.5 mg/ml diaminobenzidine (DAB, Sigma D5637), 0.4 μ l/ml 30% hydrogen peroxide. Sections were mounted from 0.1 M saline onto chrome-alum subbed slides, dehydrated, and coverslipped with glycerol-gelatin (Sigma GG-1).

Slides were examined on an Olympus BH-2 brightfield and epifluorescence microscope fitted with a Hitachi KP-D581 color digital video camera interfaced with an Integral Tech frame grabber in a desktop computer. Motorized stage and focus (Prior Scientific, Rockland, MA), as well as image acquisition, were controlled through ImagePro Plus (Media Cybernetics, Silver Springs, MD). Injection tracks were mapped to coronal sections of the Paxinos and Watson rat brain atlas [57].

2.7. Statistical analysis

Data were analyzed with SAS (version 9.1) statistical software. In all analyses, $P < 0.05$ was considered to be statistically significant. Data were analyzed as follows. Errors and perseverative errors were averaged across daily sessions (1–5) for each rat. Errors and perseverative errors committed per testing block were analyzed by two-way repeated measures ANOVA, with treatment group (tau *vs.* GFP control; between subjects), testing block (1–15; within subjects), and the interaction entered as factors.

3. Results

3.1. Histological results

Injections resulted in reproducible expression of GFP in hippocampus proper and dentate gyrus (Fig. 1). In animals that received the GFP control vector, expression at 25 weeks post-injection was observed exclusively in neurons. Expression was highest near the injection sites, and there was comparable right/left GFP expression as well as comparable expression between animals. The GFP filled neurons entirely, revealing isolated projections into the entorhinal cortex, thalamus, and cerebral cortex as well as intrahippocampal connections. Transduced neurons were found throughout the dorsal-ventral extent of the hippocampus observed at all septo-temporal levels of the hippocampal formation [37,56,57]. Numbers of neuron cell bodies ranged up to several hundred per section for both vectors.

The expression of P301L tau in rats injected with the P301L tau vector was also restricted to neurons. The antibody HT7 to human tau revealed tau throughout most of the septal-temporal extent of the hippocampus (Fig. 2B). All P301L tau vector rats exhibited human tau expression in a subset of hippocampal neurons. Antibody AT8, which recognizes tau's phosphorylated serine residue 202/205, labeled a similar but smaller population of neurons

than HT7 (Fig. 2A). Thioflavin S affinity dye and NFT antibody, which specifically labels human neurofibrillary tau tangles, however, labeled different structures than the other tau antibodies (Fig. 3A and B). Structures resembling NFTs were observed consistently in tau animals but were not abundant. However, numerous irregular punctate objects smaller than neuronal somata were located near the injection sites and in hippocampal subfields. Hippocampal tissue from rats injected with the GFP vector was blank for these markers.

Profound neuronal loss (death and atrophy) was detected in tau-expressing neurons in the tau group. Widespread phagocytic microgliosis (ED-1/CD-68 immunolabeling) (Fig. 3D) and astrogliosis (GFAP immunolabeling) (Fig. 3E) were observed apart from the typical mild inflammatory reaction along the injection needle track (Fig. 3C) that is also observed in control injections. Neurons immunoreactive for oxidized DNA (Fig. 3F) were far more abundant in tau vector brains.

3.2. Behavioral results

No gross motor abnormalities were observed at any point during testing. Two-way repeated-measures ANOVA revealed a significant main effect of treatment group ($F_{1,224} = 33.21$, $P < .0001$), with tau rats committing more errors than controls. There was also a significant main effect of testing block ($F_{14,1124} = 20.51$, $P < 0.0001$). Control rats and tau virus rats made progressively fewer errors over the course of the 15 testing blocks as expected. The interaction was not significant. Tau rats committed an average of 3.15 ± 0.30 errors per test block, compared to an average of 2.14 ± 0.34 errors per test block for controls. Fig. 4 shows that while the number of errors per trial decreased for both groups over time, the control group consistently performed better than the tau group.

For perseverative errors, tests for normally distributed data prompted a square root transformation to normalize the data because residuals were positively skewed. Two-way repeated-measures ANOVA revealed a significant effect of treatment group ($F_{1,224} = 41.81$, $P < .0001$), with tau rats committing more perseverative errors than controls. There was also a significant main effect of testing block ($F_{14,224} = 16.04$, $P < .0001$). Both GFP control rats and tau rats made progressively fewer perseverative errors over the course of the 24 testing blocks. Tau-treated rats committed an average of 1.01 ± 0.23 perseverative errors per test block, compared to an average of 0.46 ± 0.19 perseverative errors per test block for controls. Fig. 5 shows that while the number of perseverative errors per trial decreased for both groups over time, the control group consistently performed better than the tau group.

4. Discussion

Focal expression of mutated P301L tau in the hippocampus progressively impaired performance on a hippocampal-dependent spatial memory task. The learned alternation task allowed for continuous behavioral testing over six months, as opposed to a single water maze probe trial, which would have less utility for continually monitoring the progression of behavioral deficits found in our model of tauopathy. Of note is the fact that the acquisition rates for the learned alternation task were similar between the tau and GFP vector-injected rats after intrahippocampal vector injection for approximately one month, but diverged thereafter, with the tau group performing significantly worse than the GFP control rats. The similar rates of acquisition reveal that vector-induced tauopathy did not have a behavioral impact for at least one month after vector injection, presumably the time at which mutated tau expression was initiated. The time course of behavioral impairment we observed makes our tauopathy model useful for examining mechanisms leading to tauopathy and their behavioral sequelae. Beyond this, the similar acquisition rates between tau and GFP vector-injected rats during the first month are interesting given the boost in memory performance that has been documented in young P301L tau transgenic mice prior to tauopathy expression

[58]. This feature provides an additional point of similarity of our model to existing models of tauopathy, and it may have diagnostic relevance as an early biomarker of tauopathy.

Tau vector-injected rats also made more repeated errors on the task relative to the GFP vector-injected rats. At the time of sacrifice, the brains of the tau group exhibited marked hippocampal tauopathy, including extensive hyperphosphorylated tau and NFTs throughout the hippocampus. The impairment on the learned alternation task is likely a result of the degenerative consequences and dysfunction of hippocampal neurons associated with P301L tau expression. It is possible that the tau group's performance diverged from that of the GFP-treated rats starting at behavioral testing week 5 due to tau expression reaching a high level around week 4–5 post-injection. It would be important in future studies to characterize the tauopathy before and at the time at which behavior of the tau and control groups diverges (in this study, at behavioral testing week 5) [12]. The progressive development of the mnemonic deficit (Fig. 4) and pronounced perseverative behavior (Fig. 5) seen in the tau group, coupled with the low variance in erroneous responses in the behavioral task among tau-receiving animals, lends credence to the idea that the present rodent model of tauopathy is relevant for modeling progressive human dementia. It also argues for the importance of learning and memory tests that permit multiple repeated assessments, which is not feasible with many standard tasks such as water maze acquisition and retention.

Further support for the idea that the present rodent model is relevant for human dementia comes from the histological analysis. Substantial human tau protein was detected in neurons in CA1, CA2, CA3, hilus, subiculum, and dentate gyrus subregions along most of the septo-temporal extent of the hippocampus, as typically observed for comparable AAV vector injections [59]. Vector-induced tau expression led to hyperphosphorylated tau aggregates and structures resembling human NFTs. Two papers have recently demonstrated the trans-synaptic spread of human tau through the brain [60,61]. Given our discovery of tau in neighboring brain regions from the sites of viral vector injections, our data fit the neuronal system propagation events described in these papers. However, since we employed a viral vector to deliver human tau to animals, we cannot rule out viral spread (*i.e.*, distant transduction) over direct trans-neuronal propagation of tau as the mechanism by which tau spread to hippocampal regions from the sites of viral vector injection. Nonetheless, we show clear evidence of tau throughout the hippocampus, and our results support the hypothesis that an adult-onset tauopathy that is spatially restricted to the hippocampus alone can effectively produce progressive mnemonic deficits analogous to those seen in human dementing tauopathies. We have previously shown that P301L tau transduction in the entorhinal cortex of adult rats produced neither extensive neuronal loss nor extensive degeneration of axons [12]. If neither neuronal loss nor axon degeneration are observed in the presence of robust mnemonic deficits, then tau dysfunction in the hippocampus, which would impair cellular dynamics without necessarily killing the cells early in the tauopathy, could produce mnemonic deficits. In the present study, we did note neuronal degeneration, although survival extended substantially beyond the onset of behavioral deficits. Exploration of the anatomical profile of the hippocampus at different time points during behavioral testing after the mutated tau_{P301L} vector injection will be required to resolve this issue. However, the possibility that pathogenic tau or derived fragments could be transferred to non-transduced neurons connected to vector-transduced hippocampal neurons [60–62] suggests that loss of function could involve brain regions outside the hippocampus. The vector approach does not yet allow discrimination of transferred proteins from those associated with remote gene delivery.

In addition to observing cell loss in the hippocampus, we also noted gliosis associated with the expression of the tau pathology (Fig. 3). AAV-induced wild-type tau has previously been shown to cause gliosis in targeted brain regions [35,63]. Gliosis is one of the important

sequelae of mutant human AAV9_{P301L} tau expression that may relate to the behavioral findings of this study, as there was prominent gliosis in the hippocampus of tau experimental animals only (Fig. 3). Astrogliosis is typically a delayed and sustained response to neuronal injury and tissue reorganization. Microgliosis, especially with the rounded phagocytic morphology observed in tau vector brains, is likely to reflect not only recent (ongoing) scavenging of cellular debris arising from neuronal cell death but can also be a primary mechanism of neuronal loss [64]. The CD68 marker (ED-1) clearly demonstrates large numbers of cells with a phagocytic phenotype, reflecting a robust, prolonged inflammatory response [65]. This population may include not only activated microglia but infiltrating peripheral macrophages and monocytes [66].

The hippocampal contribution to spatial memory is clearly substantial [42–46]. The hippocampus contributes to both acquisition and retention of spatial alternation tasks. It is involved in the acquisition of new memories [42] as well as the retention of long-term memories [43]. Our findings support the interpretation that the hippocampus contributes to the performance of a spatial memory task. Consistent, irreversible impairments in task performance due to rAAV9-induced transduction of P301L tau were apparent as early as about 6 weeks after tau transduction (*i.e.*, about week 5 of behavioral testing) and persisted for up to 24 weeks. Thus, highly targeted tau expression in the hippocampus progressively impaired spatial working memory.

A substantial literature documents the deleterious effects of transgenic P301L tau expression on mnemonic function [24–26,36,37,39,40,67,68]. The major finding of our study is that working memory on a spatial alternation task was significantly impaired by the transduction of hippocampal neurons with P301L tau virus vector. We show here that localized somatic cell gene transfer tauopathy can produce mnemonic deficits that persist for as long as 6 months. While we have shown that mnemonic deficits in tau rats are pronounced at 6 months after tau transduction, the use of a behavioral task appropriate for repeated assessment revealed that irreversible mnemonic deficits using this tauopathy model are evident as early as 5 weeks of behavioral testing after P301L transduction. This rapid time course of functional impairment on a hippocampal-dependent memory task is a feature that makes our rodent model particularly suitable for rapid drug screening to identify potential therapeutics for tauopathies. The gene delivery approach permitted clear demonstration, dissociated from developmental and maturational confounds, that discretely localized single gene expression changes in a brain structure contributing to working memory can reproduce key behavioral and neuropathological phenotypes found in human tauopathies. Tau gene delivery provides a powerful, economical, and convenient animal model for studying tau_{P301L}'s role in synaptic dysfunction and memory.

Acknowledgments

Supported by grants from the National Institutes of Health (MH060608), the National Science Foundation (IOS-1048556) and the Howard Hughes Medical Institute (52005120) to JJR, National Institute on Aging (P10485) and the U.S. Department of Veterans Affairs to MAK, and NIH grant no. NS048450 to RLK. We thank Courtney Cron, Mimi Cushman, Anne Herzog, and Kim Lang for assistance with behavioral testing and Erik Knelson for sharing his expertise in the surgical procedures with us. The authors also wish to thank Dr. Justin Rhodes, University of Illinois at Urbana-Champaign, for his help with the statistical analyses.

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Fig. 1.

Injection of control rAAV (constructed to drive the expression of GFP) into rostro-dorsal hippocampus produced robust expression in hippocampus proper and dentate gyrus (white arrow). Top panel scale bar is 1 mm, native GFP fluorescence in low-level brightfield illumination. Panel below, reconstruction [modified from Paxinos & Watson, 1986] of case with rAAV9 constructed to drive the expression of P301L human tau. Red indicates areas in which human tau was expressed. Black indicates areas that were severely degenerated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 2.

Human tau immunoreactivity was observed in the hippocampal formation.

Immunoreactivity for hyperphosphorylated ser202/205 tau in somata, dendrites (CA3) and axons (inner molecular layer) was observed, with severe narrowing of CA1 alveus-fissure distance (line) reflecting degeneration and atrophy (antibody AT8, panel A, 4 $\times$, scale bar is 100 μ m). Immunoreactivity for human tau was located in somata and dendrites of transduced neurons (antibody HT7, black arrow, panel B, 20 $\times$, scale bar is 25 μ m).

Immunoreactivity for human tau was dense near the injection site in the caudal hippocampal formation (HT7, panel C, 4 $\times$, injection at circle) and was selectively located in neurons.

Controls were blank for human tau immunoreactivity. Scale bar is 1 mm.

Fig. 3.

(A) Thioflavin S affinity dye labeling in CA3. Large neuron (arrow) exhibited perikaryal NFT (20 $\times$). Scale bar is 25 μ m. Control sections from GFP animals did not exhibit thioflavin labeling. (B) NFT in CA3 of tau animal. Two neurons (arrows) exhibited perikaryal NFT (20 $\times$). Scale bar is 25 μ m. (C) Immunolabeling for phagocytic cells (antibody CD-68) shows activated microglia (and probably infiltrating myeloid cells) (black arrows) only along the injection track (line) of control section from GFP animal. Dorsal is to the right. Scale bar is 100 μ m. (D) Tau vector-injected rat showing far more numerous and distributed reactive microglia in hippocampus than what occurs from injection alone. Phagocytic morphology is consistent with extensive neuronal death and axonal degeneration in hippocampal fissure

(HF), CA1 pyramidal layer (CA1 SP), dentate granule cell layer (DGCL), hilus (H), and alveus (ALV). Scale bar is 100 μm . (E) Immunolabeling for astrocytes (antibody GFAP) in dentate gyrus (single arrow) and hippocampal fissure (double arrows) shows pronounced astrogliosis in a tau animal (4 $\times$). Scale bar is 100 μm . (F) 8-hydroxydeoxyguanosine immunolabeling for oxidized DNA in tau animal shows clear evidence of DNA oxidative stress in CA1 (20 $\times$). Scale bar is 25 μm .

Fig. 4.

Rats that sustained bilateral injections of rAAV9-tauP301L were significantly impaired in spatial memory testing relative to the GFP group. Acquisition rates of the spatial alternation task were similar between the tau and the GFP vector-injected rats for the first four blocks of testing (~1 month after intrahippocampal vector injection), but diverged thereafter, with the tau group committing significantly more errors ($P < 0.05$) than the GFP-injected group (error bars = $\pm$ S.E.M.). The black bar indicates the last three blocks of testing over a three month interval, during which testing occurred for one week per month.

Fig. 5.

Tau vector-injected rats displayed significant perseverative behavior on the learned alternation task relative to the GFP vector-injected rats. As depicted, rats injected with the tau vector committed consecutive incorrect responses more frequently ($P < 0.05$) than the GFP-injected group (error bars = $\pm$ S.E.M.). The black bar indicates the last three blocks of testing over a three month interval, during which testing occurred for one week per month.