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Frontotemporal lobar degeneration related proteins induce only subtle memory-related deficits when bilaterally overexpressed in the dorsal hippocampus

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Abstract

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disease that involves cognitive decline and dementia. To model the hippocampal neurodegeneration and memory-related behavioral impairment that occurs in FTLD and other tau and TDP-43 proteinopathy diseases, we used an adeno-associated virus serotype 9 (AAV9) vector to induce bilateral expression of either microtubule-associated protein tau or transactive response DNA binding protein 43 kDa (TDP-43) in adult rat dorsal hippocampus. Human wild-type forms of tau or TDP-43 were expressed. The vectors/doses were designed for moderate expression levels within neurons. Rats were evaluated for acquisition and retention in the Morris water task over 12 weeks after gene transfer. Neither vector altered acquisition performance compared to controls. In measurements of retention, there was impairment in the TDP-43 group. Histological examination revealed specific loss of dentate gyrus granule cells and concomitant gliosis proximal to the injection site in the TDP-43 group, with shrinkage of the dorsal hippocampus. Despite specific tau pathology, the tau gene transfer surprisingly did not cause obvious neuronal loss or behavioral impairment. The data demonstrate that TDP-43 produced mild behavioral impairment and hippocampal neurodegeneration in rats, whereas tau did not. The models could be of value for studying mechanisms of FTLD and other diseases with tau and TDP-43 pathology in the hippocampus including Alzheimer's disease, with relevance to early stage mild impairment.

Introduction

We studied whether two proteins related to human pathology in neurodegenerative diseases, in particular FTLD, would induce memory deficits in order to model cognitive loss caused by these protein pathologies. The vast majority of postmortem pathology in FTLD involves either neurofibrillary pathology, i.e. microtubule-associated tau pathology (FTLD-Tau), or aberrant pathology of the TDP-43 protein (FTLD-TDP; Chen-Plotkin et al., 2010; Geser et

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al., 2010). Because memory loss occurs in FTLN and other neurodegenerative diseases involving tau or TDP-43 pathology, likely as a result of hippocampal involvement to some extent (Frisoni et al., 1999; Laakso et al., 2000; Teipel et al., 2006), we addressed whether memory-related behavior of rats would be affected by targeting tau or TDP-43 expression to the hippocampus, attempting a relevant rat model of FTLN. The anterior portions of human hippocampus are particularly atrophied in FTLN (Laakso et al., 2000), and the anterior (rostrom-dorsal) hippocampus may be particularly salient for spatial memory function in rats (MacDonald et al., 2010). We selectively targeted the rostrom-dorsal hippocampus in rats.

Tau is a major pathology in Alzheimer's disease (AD) along with beta-amyloid (Wilcock and Esiri, 1982), and tau pathology is the primary pathology in several progressively dementing diseases that fall under the umbrella term of FTLN-Tau, e.g., Pick's disease, progressive supranuclear palsy and corticobasal degeneration (Chen-Plotkin et al., 2010; Geser et al., 2010). Tau neurofibrillary tangles are also found in cases of dementia related to high impact contact sports (Miller, 2009). For several reasons, tau pathology is associated with cognitive impairment. Braak staging of neuropathological progression in AD is based on tau pathology originating in the entorhinal cortex and spreading to the hippocampus and cortex with advanced age and disease (Braak and Braak, 1991). The degree of neurofibrillary pathology has been shown to correlate with the severity of AD memory-related symptoms even more than plaque pathology (Wilcock and Esiri, 1982; Arriagada et al., 1992; Bierer et al., 1995). Clinical trials targeting amyloid have been unsuccessful in improving function thus far (Abbott, 2008; Brunden et al., 2009; Extance, 2010) unfortunately, and may suggest that targeting tau could have more impact. The relationship of tau pathology to memory function has also been demonstrated in tau transgenic mouse models. For example, memory-related deficits have been reported in mice expressing the P301L form of tau, which is associated to the tauopathy disease frontotemporal dementia with parkinsonism linked to chromosome 17 (Ramsden et al., 2005; Santacruz et al., 2005; Murakami et al., 2006; Berger et al., 2007). Furthermore, it has been reported that endogenous tau expression is necessary for memory-related effects to occur in amyloid mice, because reducing tau expression prevented the memory impairments from developing (Roberson et al., 2007; Ittner et al., 2010).

Direct injections of gene transfer vectors are a complimentary approach to express tau in amyloid mice or in rats (Klein et al., 2004; Jaworski et al., 2009) because the expression can be targeted to the most relevant brain regions, avoiding other parts of the CNS, as well as other tissues. Previous studies demonstrated widespread expression that was well contained within the hippocampus with an AAV9 GFP vector (Klein et al., 2008), without damage to cortex above the hippocampus along the needle track, which enables discrete study of the relationship of hippocampal tau pathology and memory-related behavior. Tau pathology is closely linked to cognitive loss in humans and animal models, so relevant and cost-effective functional models based on tau could be appropriate for therapeutic screening. The facility to use rats or even non-human primates with the vector approach could improve functional paradigms, while another advantage is combining vector with existing transgenic lines, rather than having to cross lines to get co-expression.

Studying memory-related effects of TDP-43 expression is relevant to cognitive decline in FTLN (Neumann et al., 2006; Neumann, 2009; Chen-Plotkin et al., 2010; Geser et al., 2010). TDP-43 pathology is common in cases of hippocampal sclerosis and dementia found in the aged (Josephs & Dickson, 2007; Amador-Ortiz, 2007a) as well as in ALS, where cognitive decline may occur (Abe et al., 1997; Phukan et al., 2007; Takeda et al., 2009). TDP-43 pathology is also relatively common in AD (Amador-Ortiz et al., 2007b; Uryu et al., 2008; Arai et al., 2009). The presence of hippocampal TDP-43 lesions in these diseases was the basis for targeting TDP-43 expression to the hippocampus. Studies of TDP-43

transgenic mice have reported ALS-like spinal and motor effects (reviewed in Wang et al., 2011). Although one study was able to demonstrate specific memory impairment when expression was limited to the forebrain (Tsai et al., 2010), motor debilitation occurred later. TDP-43 expression targeted to the hippocampus could provide a relevant and cost-effective assay of neurodegeneration and memory loss isolated from potential motor impairment.

Wild type forms of human tau or TDP-43 were compared to two control groups, a GFP AAV9 vector control, as well as a vehicle buffer injection control for memory-related behaviors in the Morris water task, where the subjects learned the location of a platform, and were then tested for retention of the location later. Injection placement and vector doses were selected to achieve widespread distribution of transgene expression selectively within the dorsal hippocampus, although with a vector designed for moderate expression levels within neurons, i.e., using a weaker promoter system than used in previous studies. Cellular markers of pathology were viewed at the conclusion of the behavioral study. Specific disease-relevant memory impairment and neurodegeneration could indicate a valid assay for studying tau and TDP-43's role in diseases with cognitive loss.

Materials and methods

DNAs and AAVs

The expression cassettes used the hybrid cytomegalovirus/chicken β -actin promoter, and the bovine growth hormone poly adenylation sequence flanked by AAV2 terminal repeats. Plasmid DNAs with either GFP, human wild-type tau longest form (4 microtubule binding domain repeats including exons 2/3/10), or human wild-type TDP-43 were packaged into recombinant AAV9 vectors. In an attempt to study mild to moderate expression, we did not include an enhancer element used previously that boosts expression (woodchuck hepatitis virus post-transcriptional regulatory element, WPRE; Loeb et al., 1999; Paterna et al., 2000; Klein et al., 2006). The helper plasmids to make AAV9 were a kind gift from James Wilson and the University of Pennsylvania Gene Therapy Center (Gao et al., 2002; 2004). The AAV vector preparation used methods from Zolotukhin et al., (2002), Paterna et al., (2004), and Klein et al., (2008). Human embryonic kidney 293-T cells were co-transfected with the transgene plasmid along with two helper plasmids needed to make AAV9 by the calcium-phosphate method. After 3 days, cells were harvested, lysed, applied to a discontinuous gradient of iodixanol (OptiPrep; Greiner Bio-One, Longwood, FL), centrifuged, and washed and concentrated using Millipore (Billerica, MA) Biomax 100 Ultrafree-15 units. The virus was resuspended in the vehicle, lactated Ringer's solution (Baxter, Deerfield, IL), and filter sterilized using Millipore Millex-GV syringe filters. Vectors were aliquoted and stored frozen. Encapsidated genome copies were titered by a dot blot method.

Groups, Vector Dose, Time Points

The experiment used male Sprague-Dawley rats (approx. 12 weeks old, from Harlan, Indianapolis, IN). Tau and TDP-43 were compared to two control groups, a lactated Ringer's vehicle control, and a GFP control AAV9 vector. Equal dose comparisons were made across all vector groups by normalizing titers with the diluent, lactated Ringer's solution. Injections to the hippocampus were bilateral and used a total dose of 1×10^{10} vector genomes per side. This dose was chosen from earlier experiments that achieved widespread GFP expression in the hippocampus (Klein et al., 2006; 2008). The vectors were coded prior to injections to establish blind conditions throughout the entire experiment including postmortem data collection. A 7 week period was chosen to allow for maximal expression after gene transfer (Klein et al., 2009), and then the animals performed the training phase of the Morris water task to learn the location of the platform. The animals were again tested 12 weeks later in the retention phase. Histological analyses began 1 week after the behavioral tests, and were

therefore at approximately 32 weeks of age, which was 20 weeks after gene transfer. There were 6 rats in the vehicle control and TDP-43 groups and 11 and 12 rats in the GFP and tau groups, respectively. We ran more tau (and GFP control) rats to increase statistical power based on initial trends observed in a smaller tau group, whereas treatment effects were detectable in the small TDP-43 group. With two injections per rat, there were 70 stereotaxic injections analyzed in the study.

Stereotaxic injections

Subjects were anesthetized with a cocktail of 3 ml xylazine (20 mg/ml, from Butler, Columbus, OH), 3 ml ketamine (100 mg/ml, from Fort Dodge Animal Health, Fort Dodge, IA), and 1 ml acepromazine (10 mg/ml, from Boehringer Ingelheim, St. Joseph, MO) administered intramuscularly at a dose of 1 ml/kg. Viral stocks or lactated Ringer's vehicle were injected through a 27 gauge cannula connected via 26 gauge internal diameter polyethylene tubing to a 10 μ l Hamilton syringe mounted to a microinjection pump (CMA/Microdialysis, North Chelmsford, MA) at a rate of 0.2 μ l/min. Stereotaxic injection coordinates for the dorsal hippocampus were 3.6 mm posterior to Bregma, 2.0 mm lateral, and at two depths, 3.5 and 2.8 mm ventral, with 3 μ l injected at each depth (Paxinos and Watson, 1998). The needle remained in place at each injection site for 5 additional minutes before being slowly withdrawn. The skin was sutured, and the animal was placed on a heating pad until it began to recover, before being returned to its individual cage. All animal care and procedures were in accordance with Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Morris Water Maze

The rats were tested for memory-related behavior using the Morris water maze (Morris, 1984). A platform (15 cm $\times$ 15 cm) was placed in one location, in the center of one quadrant of a large circular tank (183 cm diameter by 61 cm deep). The water level of the tank was maintained at 3 cm above the platform. The tank was filled at least one day prior to testing to allow the water to adjust to the ambient room temperature of 22°C. Animals were placed on the platform for 10 sec prior to the first trial on the first day for orientation. The acquisition phase consisted of 5 days of testing with 4 trials per day. For each trial, the rat was placed in 4 different specific starting locations 90° apart. Each rat was given up to 60 sec to find the platform. If unable to find the platform, the rat was guided onto the platform, where it remained for 10 sec. Then the rat was removed from the tank, patted dry, and placed in a drying cage for 5–10 min. The time interval between trials on each day was 20 min. The retention phase was 12 weeks after the acquisition phase and consisted of one 60 sec run without the platform. Rats were placed in the tank from a fixed location for the retention test. Latency to the target location, time spent in the target zone (tank divided into 12 zones), and the number of target entries (crossings) were recorded using the SMART v. 2.5.12 video tracking system (San Diego Instruments, San Diego, CA). The 12 zones were derived from the 4 quadrants with 3 concentric rings. Rest time was defined as time spent moving less than 2 cm/sec. Thigmotaxis was defined as time spent in an outer ring zone 17 cm from the wall.

Locomotor activity

At 10 weeks after gene transfer, and 3 weeks after the training in the Morris water task, rats were tested for total locomotor activity as a control for specificity for a memory-related effect. Rats were placed in a 18 $\times$ 18 $\times$ 18 inch enclosure (Tru-Scan, Coulbourn Instruments, Whitehall, PA) for 30 min in the dark and total distance travelled was computed.

Histological staining

Anesthetized animals were perfused with PBS, followed by cold 4% paraformaldehyde in PBS. The brain was removed and immersed in fixative overnight at 4 °C. Brains were equilibrated in a cryoprotectant solution of 30% sucrose/PBS at 4°C for 2–3 days. Coronal sections (50 µm thick) were cut on a sliding microtome with a freezing stage. Primary antibody incubations on free-floating sections were kept overnight at 4° C on a shaking platform. Primary antibodies for immunostaining included the following: human-specific TDP-43 antibody (1:1000; Abnova, Taipei City, Taiwan); glial fibrillary acidic protein (1:400; Chemicon) for astroglia, CD11b (1:400; Chemicon, Millipore, Billerica) for microglia, Neuronal nuclei (NeuN) for neurons (1:500; Chemicon), GFP (1:10,000; Molecular Probes/Invitrogen, Carlsbad, CA), E1 for detecting human specific tau (1:2000; a gift from Leonard Petrucelli, Mayo Clinic, Jacksonville, FL; Crowe et al., 1991), CP13 for hyperphosphorylated tau at serine 202 (1:500; a gift from Peter Davies, Albert Einstein College of Medicine, Bronx, NY; Jicha et al., 1999), Ab39 for mature neurofibrillary pathology (1:100, a gift from Shu-Hui Yen, Mayo Clinic, Jacksonville, FL; Yen et al., 1985). For immunoperoxidase staining, endogenous peroxidase activity was quenched with 0.1% H₂O₂/PBS for 10 min. The sections were washed in PBS and incubated for 5 minutes in 0.3% Triton X-100/PBS, and washed before applying primary antibody. Biotinylated secondary antibodies for peroxidase staining were from DAKO Cytomation (1:2,000; Carpinteria, CA), incubated for 1 hour at room temperature. The sections were washed with PBS and labeled with horseradish peroxidase-conjugated Extravidin (1:1000; Sigma, St Louis, MO) for 30 minutes at room temperature. The chromogen was diaminobenzidine (0.67 mg; Sigma) in 0.3% H₂O₂, 80 mmol/l sodium acetate buffer containing 8 mmol/l imidazole and 2% NiSO₄. After mounting on slides, the sections were dehydrated in a series of alcohol and xylene and coverslipped with Eukitt (Electron Microscopy Sciences, Hatfield, PA). For immunofluorescence, sections were incubated in primary antibody overnight, washed and incubated with either Alexa Fluor 488 (Invitrogen) or Cy3-conjugated secondary antibodies (1:300; Jackson ImmunoResearch, West Grove, PA) for 2 hours, followed by DAPI counterstaining (1 µg/ml; Sigma, St. Louis, MO), washing, and coverslipping with glycerol/gelatin (Sigma). For Nissl staining, a 0.5% cresyl violet (Acros Organics, Fairlawn, NJ) solution for 25 min was used as described in Paxinos and Watson (1998). The slides were then washed in water and dehydrated in a series of alcohols and xylene and coverslipped with Eukitt.

Hippocampal size measurements

Measuring hippocampal volume is clinically relevant for neurodegenerative and neurological diseases, as well as normal aging (Josephs & Dickson, 2007; Amador-Ortiz, 2007a; Sicotte et al., 2008; Aylward et al., 1999; Driscoll et al., 2003). The volume of the dorsal part of the hippocampus, from 2.3 to 4.4 mm posterior to Bregma (Paxinos and Watson, 1998) was estimated from 7 evenly spaced sections stained for Nissl substance. The unbiased Cavalieri method on the StereoInvestigator program from MicroBrightfield Inc. (Williston, VT) was used to estimate tissue volumes. Each bilateral injection was considered separately, i.e., two values from each subject. The hippocampus/dentate gyrus was outlined on each section, and grids were randomly oriented and spaced to yield ca. 500–600 markers. Markers interior to the traced boundaries were counted automatically, and the volume of the region of interest was estimated from the number and spatial frequency of the markers, section thickness, and section sampling frequency. Error values were estimated at less than 0.02 for each measurement. The thickness of layers in the hippocampus were also measured using the StereoInvestigator program, from CA1 pyramidal cells to the granule cells in the dorsal blade of the dentate gyrus. Three sections were analyzed centering the injection, and on each section, 3 evenly spaced lines were measured starting on the Nissl stained cells in

the CA1 pyramidal layer and ending at the granule cells of the dorsal blade, for a total of 9 measurements that were averaged for each rat.

Statistics

Data are expressed as mean $\pm$ SEM. Statistical tests were repeated measures ANOVA or ANOVA with Bonferroni multiple comparison post-tests as indicated.

Results

Behavioral effects of tau or TDP-43

The learning curves to find the platform in a Morris water task (Morris, 1984) over 5 days (Fig. 1) were compared by repeated measures ANOVA to evaluate the effect of vector, time interval, and the interaction. A main effect of time ($F(4, 31) = 36.32$, $P < 0.0001$, $N = 6-12$ per group) corresponded to task learning, though there were no significant group differences or interaction. Twelve weeks after the acquisition phase, memory for the platform location was tested a single probe trial with the platform removed from the chamber (Fig. 2). ANOVAs revealed an effect of vector group for latency to find the target location ($F(3, 34) = 3.41$, $P = 0.03$), and for target entries, i.e., number of times crossing the target location ($F(3, 34) = 3.18$, $P = 0.04$), which suggested there were specific vector group differences in performance, though the ANOVA for time in target zone was not significant. Bonferroni multiple comparisons yielded one specific group-to-group difference: the TDP-43 group had longer latency to target on average than the GFP group ($P < 0.05$), suggesting impairment. A trend of impairment was also suggested for target entries with either tau ($P = 0.017$) or TDP-43 ($P = 0.056$) relative to GFP in individual t-tests, though not significant in multiple comparisons.

The rats swam continuously during trials and had very low rest times, and there was no indication for vector group differences in wall-seeking behavior, or thigmotaxis. During the 60 sec probe phase, rest times were 1.0 ± 0.2 sec for the vehicle group, 0.6 ± 0.2 sec for the GFP group, 0.8 ± 0.3 sec for the tau group, and 1.0 ± 0.4 sec for the TDP-43 group ($N = 6-12$ /group). During the 60 sec probe phase, the times spent near the wall were 8.9 ± 2.0 sec for the vehicle group, 9.9 ± 1.1 sec for the GFP group, 11.6 ± 1.6 sec for the tau group, and 9.5 ± 4.4 sec for the TDP-43 group ($N = 6-12$ /group). The lack of differences in thigmotaxis is consistent with a specific spatial learning effect for latency to target in the TDP-43 rats.

The impairment in TDP-43 rats for latency to target was not attributed to motor dysfunction, because there were no differences in two assessments of motor function. Locomotor activity was tested at 10 weeks after gene transfer without any group differences for distance travelled in 30 min: Vehicle, 7198 ± 1060 cm; GFP, 5907 ± 991 cm; tau, 7393 ± 817 cm; TDP-43, 5857 ± 1159 cm; $N = 5-6$ /group. Swim distances during the 60 sec retention test were also not different among the groups: Vehicle, 1580 ± 97 cm; GFP, 1628 ± 77 cm; tau, 1616 ± 65 cm; TDP-43, 1599 ± 91 cm; $N = 6-12$ /group. There were also no differences in body mass across groups at the end of the experiment. Widespread TDP-43 expression in the CNS does cause paralysis and severe weight loss (Wang et al., 2010), but the focal hippocampal TDP-43 expression in this study did not produce any such obvious physiological changes or morbidity.

Expression in the hippocampus and histological effects

The histological analyses were conducted on brains harvested at 20 weeks after gene transfer, when rats were approximately 32 weeks of age. For GFP, well targeted and efficient expression was confirmed by viewing low magnification of the brain sections (Fig. 3A), with highly specific immunostaining only in the hippocampus on both sides, without

needle track damage, or extensive GFP expression in the cortex above. Widespread expression in neurons of the CA1 pyramidal cell layer is shown in Fig. 3B, also with GFP expressing neurons in the dentate gyrus (Fig. 3B). However, there were surprisingly more GFP expressing glia than expected from using these vectors unilaterally before (Klein et al., 2006; 2008). The degree of glial transduction was not quantified, but was obviously more than previous unilateral injections which produced extremely rare or no expression in glia. Non-neuronal cells are apparent by their size and shape (Fig. 3 C, D), and were consistently found in the molecular layers of the dentate gyrus and the stratum lacunosum molecular in the GFP group. Co-labeling of GFP and glial fibrillary acidic protein determined that some of these GFP positive cells were astroglia (not shown). There is an extensive anterograde cross-hippocampus commissural pathway that can be visualized on the contralateral side of a unilateral GFP vector injection (Klein et al., 2006). It is possible the contralateral projections from one side to the other resulted in concentrated levels of recombinant vector, and later, of GFP expression that could have been more likely to transduce glia, as well as to activate gliosis, than with previous unilateral injections. The GFP detection in glia means these cells were transduced, but could also be related to activation of these cells (Peel and Klein, 2000). The glial transduction was unexpected, and presumably occurred in the other vector groups, although tau or TDP-43 labeling did not clearly visualize non-neuronal cells as GFP did. The transduction patterns were consistent for bilateral expression and spread of specific gene expression for every injection. However, given the large volume of the hippocampus, clearly there was incomplete transduction of all the neurons in the entire region. In particular, the injections were unsuccessful in transducing the posterior/ventral (caudo-ventral) regions of the hippocampus (> 5 mm posterior to Bregma, data not shown), with only the dorsal part of the hippocampus efficiently transduced.

Similar to the GFP, staining for the human tau transgene product was impressive at low magnification in terms of its spread and selectivity in the hippocampus (Fig. 4A). However, the tau immunoreactivity was dense in the neuropil, which shrouded visualization of neuronal perikarya. The tau immunolabeling was not observed in non-neuronal cells as seen in the GFP group, which could be due to differences of intracellular distribution of the two markers. Neuronal cells could be discerned with antibodies for pathological and hyperphosphorylated tau (Jicha et al., 1999; Fig. 4D) and neurofibrillary tau (Yen et al., 1985; Fig. 4E). The three tau antibodies used suggested detection of specific pathologies because the hyperphosphorylated tau (CP13) immunoreactivity pattern was a subset of total human tau (E1), and the subset pattern for neurofibrillar tau (Ab39) was even smaller.

Human TDP-43 staining was also highly specific, and the expression was unequivocally in nuclei (Fig. 5). We did not detect TDP-43 deposition in neuronal cytoplasm as occurs in TDP-43 diseases (Neumann et al., 2006). The nuclear pattern with TDP-43 is not visible on whole sections as in Figs. 3A and 4A, because of the lack of neuropil staining, but did demonstrate widespread expression in CA1, CA3 and dentate gyrus granule cells at higher magnification (Fig. 5A–C). The nuclear TDP-43 staining pattern suggested neuronal loss in granule cell layer of the dorsal blade of the dentate gyrus (arrow in Fig. 5A) that was confirmed by the other staining methods.

To visualize hippocampal neurons for all of the groups, we stained for Nissl substance (Fig. 6). Staining patterns were similar in the vehicle control, GFP, and tau groups. There was a consistent loss of granule neurons in the dorsal blade of the dentate gyrus only in the TDP-43 group, which was observed in all of the TDP-43 animals. Nissl analysis was confirmed with the neuronal marker NeuN, which mirrored the pattern of consistent neuronal loss only in the TDP-43 group (not shown). The dorsal part of the hippocampus was analyzed for tissue volume, which agreed with the observed cell loss. ANOVA/ Bonferroni tests revealed significant volume shrinkage for the TDP-43 group vs. the vehicle

control or GFP vector control ($P < 0.01$; $N = 10$ – 24 per group; Fig. 7A). The thickness of the hippocampal layers near the injection site was reduced in the TDP-43 rats relative to the two control groups, and also when compared to the tau group ($P < 0.001$ – 0.01 ; $N = 10$ – 24 per group; Fig. 7B).

Previous time course studies with a control AAV9 GFP vector have shown an initial increase in microglial staining within 1 week after the injection, followed by attenuation (Tatom et al., 2009). As expected, at 20 weeks after gene transfer, neither the control vehicle injections nor the control GFP injections showed pronounced microgliosis, despite abundant GFP expression in the latter group (Fig. 8A, B). A similar absence of microgliosis was observed in the tau vector group (Fig. 8C). The peak needle track damage was modest at this interval as shown for several representative animals (Fig. 8A–C). However, there was obvious and consistently elevated microglial immunoreactivity (CD11b) near needle tracks of the TDP-43 injections, on a qualitative basis. Increased microglial immunoreactivity was most prominent in the dorsal blade of the dentate gyrus where there was neuronal loss (Fig. 8D). Upon viewing microglia at higher magnification, there were clear examples of activated microglial morphology in the TDP-43 group (data not shown), based on Kreutzberg (1996). For astroglial staining, all of the AAV vector groups had more noticeable needle scars than the vehicle injections, which were barely visible (Fig. 8E–H). As with microglial, there was a qualitative trend for elevated astroglial immunoreactivity in TDP-43 rats compared to the other groups.

Discussion

We attempted to mimic cognitive impairment in human diseases by bilateral expression of either tau or TDP-43 in the rat dorsal hippocampus. Because of the possibility that robust expression and gliosis in the GFP control vector group could affect memory-related behaviors, we ran the vehicle control group. Despite widespread neuronal GFP expression and more frequent GFP expression in non-neuronal cells than expected, the GFP vector group served as an effective control group because behavioral and cellular measures were unaffected relative to the vehicle control. There were specific effects observed only after TDP-43 gene transfer including slight, but significantly impaired memory-related behavior, shrinkage of the hippocampus, localized loss of dentate gyrus granule neurons, and microgliosis. While the amplitude of the effects in this study were minor, studying models with subtle decline in cognitive function could potentially be more relevant than models with more severe lesioning of the hippocampus, e.g. ablation (Tseng et al., 2009) or an excitotoxin (Jarrard, 2002). Therapeutic strategies may also be more relevant in the context of mild impairment when there is more restorative capacity.

There was only a trend for deficits in the tau group, which was disappointing because we predicted widespread and well targeted tau gene transfer to the rat dorsal hippocampus would produce robust neurodegeneration in addition to specific tau pathology. Restricting the transduction to neurons in the rostradorsal hippocampus may have spared sufficient hippocampal neurons in the caudo-ventral region to enable spatial memory function. The recent Loureiro et al. (2011) study suggests that the caudo-ventral hippocampus contributes to spatial memory. The non-transduced ventral hippocampus in this study may have been capable of supporting spatial memory performance in the water maze. Expression in the caudo-ventral hippocampus may have been necessary to affect memory-related behaviors after tau gene transfer, and presumably would have yielded to a more pronounced effect of TDP-43 gene transfer. In tau transgenic mice with memory deficits, there is likely more complete dorsal and ventral hippocampal expression. Tau vector expression that was well confined to the entorhinal cortex of rats impaired spatial memory function (Ramirez et al., 2011), more sensitively than the dorsal hippocampus expression in this study. In addition to

the locus of the transduction, several other differences exist between the two studies that may account for the differing results, including a different behavioral assay (spatial alternation), mutant P301L tau vs. wild type, and promoter system (WPRE enhancer). Preliminary work suggests more complete dorsal and ventral P301L tau expression in the hippocampus produces persistent spatial memory deficits on a learned alternation task (Mustroph and Ramirez, unpublished).

Though there were clinically relevant signs of tau pathology in the tau vector group, in the TDP-43 group, the human TDP-43 expression detected was predominantly in nuclei, so clinically relevant cytoplasmic TDP-43 deposits (Neumann et al., 2006) were not frequently achieved if at all in this study. However, the nuclear overexpression induced neurodegeneration and a functional defect. We assume that nuclear TDP-43 function was affected, with TDP-43 dysfunction in the nucleus causing the hippocampal neurodegeneration in this study, and the neurodegeneration in many animal studies after overexpression of TDP-43 in the nucleus (reviewed in Wang et al., 2011). Both memory retention and hippocampal volumes were subtly affected only in the TDP-43 group. The area of the hippocampus with the most noticeable loss of neurons was the granule cell layer of the dorsal blade of the dentate gyrus. As this region receives input from the entorhinal cortex via the perforant path in hippocampal circuitry, we assume loss of these cells in TDP-43 rats disrupted memory function. Without effects in the vehicle, GFP, and tau groups, there was little reason to study earlier time points. One weakness of the study, however, is not knowing the timing of the cellular effects caused by TDP-43, if they occurred early or only when memory retention was assayed later, which would require more study. The effect of hippocampal TDP-43 expression on behavior in rats was relatively minor compared to transgenic mice with TDP-43 overexpressed in the cortex and hippocampus in which acquisition of the Morris water task was nearly completely impaired at 2 months of age, before severe motor debilitation developed later as occurs in many TDP-43 models (Tsai et al., 2010; Wang et al., 2011).

Microgliosis is involved in FTLN (Ishizawa et al., 2000; Yoshiyama et al., 2007). The presence of elevated microglial staining in the TDP-43 group, and absence in the other groups demonstrates that microgliosis accompanies the neurodegeneration. We previously observed dopaminergic neurodegeneration in the rat substantia nigra using the same wild type tau vector without the WPRE expression enhancer at a similar vector dose of AAV9 (Klein et al., 2010), but hippocampal neurons were more resistant to tau expression in this study. Similar to the results with tau, pathological effects of TDP-43 were more severe in the substantia nigra using the same vector at a similar dose (Tatom et al., 2009). The different outcomes in the two regions suggest greater vulnerability of neurons in the substantia nigra, although we cannot rule out more technical explanations such as differences in vector diffusion away from the needle track.

The data suggest small changes in memory-related behavior, perhaps related to the early mild stage cognitive loss in diseases involving tau or TDP-43 pathology, such as FTLN and AD. Using aged animals, or transducing the ventral hippocampus could make more impact on behavior and be more relevant. Memory deficits in transgenic mice may take a long time to develop, so it would be valuable to have a more rapid assay for relevant cognitive decline.

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Highlights

- Two proteins related to dementia were overexpressed and tested for memory deficits.
- TDP-43 gene transfer, but not tau resulted in a memory retention deficit.
- Hippocampal loss and gliosis occurred in the TDP-43 rats.
- The vector gene transfer models may be useful to study relevant mild impairment.

Figure 1.

Morris water maze acquisition of platform location 7 weeks after gene transfer. Rats were run on 5 consecutive days with 4 trials per day. The average for the 4 trials per day is shown. Repeated measures analysis of the learning curves showed a main effect of time ($P < 0.0001$, $N = 6-12/\text{group}$), but no vector group effects or interaction.

Figure 2.

Morris water maze retention of platform location 12 weeks after training, during a 60 sec trial with the platform removed. A) Latency to platform location. B) Time spent in correct zone containing the platform (tank divided into 12 zones). C) Target entries to the exact location of the platform. For latency to find the platform, TDP-43 rats were significantly slower compared to the GFP vector control ($P < 0.05$) comparing all groups. For target entries, there were trends of deficit in both the tau and TDP-43 groups (see Results).

Figure 3.

Green fluorescent protein expression 20 weeks after gene transfer. A) Bilateral injections produced consistently widespread specific GFP immunoreactivity that was well targeted to the hippocampus and did not damage or produce extensive expression in the overlying cortex. Two serial sections from one rat. B) Widespread expression in CA1 neurons, but also non-neuronal expression in the hippocampus. CA1 pyramidal neurons; M, molecular layers of dentate gyrus; G, granule cell layers of dentate gyrus. C) Arrow, non-neuronal cell expressing GFP in the molecular layer. D) CA3 neurons expressing GFP. Bars: A = 2.6 mm; B = 268 μ m; C = 134 μ m; D = 21 μ m.

Figure 4.

Human pathological tau expression. A) Human-specific tau immunoreactivity with the E1 antibody was specifically targeted to the hippocampus on both sides. B) However, there was extensive neuropil staining due to reciprocal cross-hippocampal anterograde projections which shrouded neuronal perikarya with the E1 antibody. C, D) The CP13 antibody confirmed tau hyperphosphorylation in CA1 and CA3 neurons. E) The Ab39 antibody suggested mature neurofibrillary pathology in some cells. Controls sections from the GFP group were blank for E1, CP13, or Ab39 immunoreactivity. Bars: A = 2.6 mm; B = 335 μ m; C–E, = 21 μ m.

Figure 5. Human TDP-43 expression. A, B) Pyramidal and granule cell neuronal layers displayed strong nuclear immunoreactivity for human TDP-43, and the distinct absence in some areas such as the dorsal blade of the dentate gyrus (arrow in A) suggested neuronal loss. C) CA1 neurons expressing human TDP-43 in the nucleus. D) Control sections from the GFP group stained with the human specific TDP-43 antibody were blank for immunoreactivity. Bars: A, D = 335 μ m; B = 134 μ m; C = 21 μ m.

Figure 6.

Neuronal profiles in hippocampus 20 weeks after gene transfer. A–D) Nissl staining from each group vehicle control, GFP, tau, TDP-43. Labels in A: CA1, pyramidal cell layer; SR, stratum radiatum; SM, stratum lacunosum moleculare; M, molecular layer of dentate gyrus; D, dorsal and V, ventral blade granule cell layers of dentate gyrus. Neither GFP nor tau resulted in discernable changes relative to vehicle control. In contrast, TDP-43 rats had consistent thinning of the dorsal blade of the dentate gyrus (arrow) not found in the other groups, and consistent shrinkage of the hippocampal layers, between the pyramidal and dorsal blade granule cell layers. The vertical line in A is an example of the layer thickness measurements in Fig. 7B. Bar = 134 μ m

Figure 7.

Hippocampal size measurements. A) There was a significant shrinkage of hippocampal tissue in the TDP-43 rats compared either to vehicle control or GFP vector control, in multiple comparison tests ($P < 0.01$, $N = 10\text{--}24/\text{group}$, see Results). B) When targeting a more limited area around the needle track and measuring the thickness between the pyramidal and granular cell layers, there was a similar pattern, with shrinkage in the TDP-43 group compared to all the other groups ($P < 0.001\text{--}0.01$).

Figure 8.

Glial staining. Sections with the peak needle track damage for each animal are shown. Needle track damage was limited at 20 weeks after gene transfer. A–D) CD11b immunoreactivity was used to visualize microglia. There was no indication for microgliosis in the vehicle, control GFP vector, or tau vector groups. Relative to the other groups, TDP-43 rats did have a consistent elevation of microglial staining, in the area where there was neuronal loss, the dorsal blade of the dentate gyrus. E–H) Glial fibrillary acidic protein immunoreactivity was used to visualize astroglia. In contrast to microglial staining, all of the AAV vector groups appeared to increase astroglial staining relative to vehicle. Bar = 134 μm .