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Age-related decline in white matter integrity in a mouse model of tauopathy: an in vivo diffusion tensor magnetic resonance imaging study

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Abstract

Elevated expression of human hyperphosphorylated tau is associated with neuronal loss and white matter (WM) pathology in Alzheimer's disease (AD) and related neurodegenerative disorders. Using in vivo diffusion tensor magnetic resonance imaging (DT-MRI) at 11.1 Tesla we measured age-related alterations in WM diffusion anisotropy indices in a mouse model of human tauopathy (rTg4510) and nontransgenic (nonTg) control mice at the age of 2.5, 4.5, and 8 months. Similar to previous DT-MRI studies in AD subjects, 8-month-old rTg4510 mice showed lower fractional anisotropy (FA) values in WM structures than nonTg. The low WM FA in rTg4510 mice was observed in the genu and splenium of the corpus callosum, anterior commissure, fimbria, and internal capsule and was associated with a higher radial diffusivity than nonTg. Interestingly, rTg4510 mice showed lower estimates for the mode of anisotropy than controls at 2.5 months suggesting that changes in this diffusivity metric are detectable at an early stage preceding severe tauopathy. Immunogold electron microscopy partly supports our diffusion tensor imaging findings. At the age of 4 months, rTg4510 mice show axonal tau inclusions and unmyelinated processes. At later ages (12 months and 14 months) we observed inclusions in myelin sheath, axons, and unmyelinated processes, and a "disorganized" pattern of myelinated fiber arrangement with enlarged inter-axonal spaces in rTg4510 but not in nonTg mice. Our data support a role for

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the progression of tau pathology in reduced WM integrity measured by DT-MRI. Further in vivo DT-MRI studies in the rTg4510 mouse should help better discern the detailed mechanisms of reduced FA and anisotropy mode, and the specific role of tau during neurodegeneration.

Keywords

Tauopathy; Neurodegenerative disease; Alzheimer's disease; FTDP-17; rTg4510; Diffusion tensor MRI; White matter integrity; Electron microscopy; Ultrastructure

1. Introduction

Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism linked to tau gene on chromosome 17 (FTDP-17-Tau), and other related neurodegenerative disorders are defined by a substantial age-related decline in memory and cognitive functions that eventually include life-limiting sensory and motor impairments and co-morbid psychiatric conditions as the underlying neuropathology progresses. The presence of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) are 2 of the distinctive postmortem features of AD. In addition, there is mounting experimental support for a role of tau in white matter (WM) degeneration during the early etiology of AD (Amlie et al., 2013; Bartzokis et al., 2004; Hertz et al., 2013) and tauopathy mouse models (Lin et al., 2005; Zehr et al., 2004). Tau protein is enriched in healthy axons (Goedert, 2004), but a phosphorylated tau species that translocates to the cell soma, dendrites, synaptic terminals, and glial cells increases in AD (Janke et al., 1996) and is associated with destructive cell loss (Kowall and Kosik, 1987). Increased hyperphosphorylated tau is one of the factors involved in axonal microtubule destabilization, possibly disrupting axonal transport mechanisms (Smith et al., 2010). However, confirming whether or not this is the case, especially at early stages of AD, has relied primarily on postmortem examination or the biochemical analysis of harvested brain tissue from animal models of this neurodegenerative disease.

Diffusion tensor magnetic resonance imaging (DT-MRI) has provided significant insight into age- and AD-associated reductions in WM integrity (Bozzali et al., 2001; Medina et al., 2006; Mielke et al., 2009; Serra et al., 2010; Teipel et al., 2010). A persistent finding across reports is that fractional anisotropy (FA), one of the DT-MRI indices of the directionality of water diffusion (Pierpaoli and Basser, 1996), is reduced with age, and severely so in AD (Douaud et al., 2011). FA reductions have been shown to occur in association with increases in radial diffusivity (D_R) and mean diffusivity (D_{ave}), and reductions in the mode of anisotropy (A_{MO}) (Bozzali et al., 2001; Douaud et al., 2011, 2013). Microstructural damage as a result of demyelination, microtubule "disorganization" within axons and/or atrophy of WM tracts with resultant reactive astrocytic gliosis (Brun and Englund, 1986) may contribute the findings from DT-MRI. A highly tortuous cellular and subcellular environment is expected to alter water diffusivity and its diffusion path through the imaged tissue (Peled, 2007). Interestingly, early signs of such pathologic processes have been measured by DT-MRI in major WM structures such as the corpus callosum (genu and splenium), cingulum bundle, uncinate fasciculus (Amlie et al., 2013; Douaud et al., 2011; McMillan et al., 2013). A series of recent studies have provided evidence that reduced WM FA in AD patients correlates with cerebral spinal fluid (CSF) concentrations of the AD

throughout the entire setup and imaging session to maintain stable respiration rates. Mice were placed prone on a custom-made plastic bed with a respiratory pad placed underneath the abdomen. Respiratory rates were monitored continuously and maintained between 30–40 beats per minute by adjusting isoflurane levels. Core body temperature was maintained at 37 °C–38 °C using a warm water recirculation system (SA Instruments, Inc, NY, USA).

Anatomic scans were collected with a 2D gradient recalled echo sequence with the following parameters: 256^2 in plane by 12 axial slices, field of view was $19.2 \text{ mm}^2 \times 0.75 \text{ mm}$, flip angle 20° , number of averages were 6, echo time = 3.8 ms and repetition time = 100 ms. Diffusion weighted scans were acquired with an 8-shot spin echo planar imaging (EPI) sequence with a repetition time = 2500 ms and echo time = 24 ms. Localized whole brain voxel shimming was done (full width at half maximum linewidth ranged from 30 Hz to 70 Hz) and optimization of gradient delays was performed before each acquisition. Additional reference acquisitions were collected to correct distortions during EPI image reconstruction. The following acquisition parameters were used: data points 128^2 in plane by 12 axial slices, field of view was $19.2 \text{ mm}^2 \times 0.75 \text{ mm}$ ($150 \mu\text{m}^2$ in plane resolution), gradient amplitude 29.7 Gauss/cm, with a radio frequency pulse duration (δ) 4 ms (90° sinc excitation and 180° Mao refocusing pulse), gradient duration () 10 ms, maximum b -value 900 s/mm^2 , with a 42 direction icosahedral sampling scheme (sampled on the half sphere), and 6 B0 images (interleaved within every 7 diffusion-sensitized scans). Total scan time for 3 averages was 1 hour 40 minutes per mouse. We ran a control study on a phantom containing 1 M mannitol and 0.1 M creatine in distilled water to examine the level of EPI ghosting and distortions, motion and Eddy current artifacts with the same parameters used in our experiments (Supplementary Fig. 1). The results from this control run indicate that artifacts, especially motion-related artifacts, are minimal. Directionality or high diffusion anisotropy was not noted in the phantom and the FA value for a sampled phantom region of interest was low (between 0.08–1.0; mean diffusivity = $1267.0 \mu\text{m}^2/\text{s}$ for the same sampled region; Supplementary Fig. 1, shows a comparison to mouse brain maps).

2.3. Tensor fitting and computation of anisotropy indices

Images were corrected for motion using affine registration to a reference volume (first B0 image). Individual masks were manually generated using a B0 image and served to remove non-brain voxels. Tensor element reconstruction and estimates of 1st, 2nd, and 3rd eigenvectors and eigenvalues ($\lambda_1, \lambda_2, \lambda_3$, where λ_1 is considered a measure of axial diffusivity, or D_{Ax}), was performed using a weighted least squares fit regression on the *dtifit* program on FMRIB Software Library version 5.0 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Voxel-wise calculations of additional diffusion anisotropy indices and diffusion shape measures were carried out from the tensor element output of FMRIB Software Library. These included D_{ave} , D_R ($\lambda_2 + \lambda_3/2$), FA index, and A_{MO} (Ennis and Kindlmann, 2006; Kingsley, 2006; Pierpaoli and Basser, 1996). Examples of scalar and eigenvector maps are shown in Supplementary Fig. 1. FA varies from 0 for isotropic diffusion to 1 for full anisotropy, whereas A_{MO} describes the form that anisotropic diffusion takes. A_{MO} ranges quantitatively from -1 for a planar or “pancake-like” diffusion ellipsoid (expected from regions containing crossing fibers for diffusion in 2 dimensions) to a value of $+1$ for a linear or “cigar-like” ellipsoid expected from predominantly single fiber tract areas (Douaud et al.,

2011, 2013). An initial quantitative examination of these values in WM, gray matter (GM), and CSF of adult nonTg mice showed consistency between FA and values A_{MO} (Supplementary Fig. 2). WM (internal capsule) A_{MO} values in WM were significantly greater than in gray matter and CSF and near +1 (Supplementary Fig. 2). Diffusivity indices of these same structures were consistent with previous in vivo high field DT-MRI studies of mouse brain (Boska et al., 2007; Nair et al., 2005) (Supplementary Fig. 3).

2.4. Region of interest (ROI) analysis

Region of interest (ROI)-based analysis was used. ROI similar to those shown in Fig. 2A were manually drawn on directionally color-coded FA maps (generated using the 1st eigenvector) and compared with an atlas of the mouse brain. These included: anterior commissure, corpus callosum (CC), genu (gCC), splenium (sCC), striatum, frontal cortex (CTX), whole hippocampus, fimbria (Fmb), amygdala, internal capsule (IC), and substantia nigra (SN). ROI values for FA, A_{MO} , D_{Ax} , D_{ave} , and D_R were exported in spreadsheet form and statistical analysis was performed on Graphpad Prism using a 2 factor analysis of variance (ANOVA) with Tukey or Sidak multiple comparison test (mouse strain and age as independent variables; significance level $p < 0.05$).

2.5. Electron microscopy and post-embedding immunogold electron microscopy

Two rTg4510 mice each at the age of 4 and 12 months, and one each of nonTg mice at 12 and 14-month-old were perfused with 4% paraformaldehyde-0.1 M phosphate buffer and areas containing cortex and corpus callosum were collected and processed for regular and immunoelectron microscopy (IEM). The method has been used in previous publications (Lin et al., 2003; Ren et al., 2013).

For IEM, tissues were dehydrated in 30%, 50%, 70%, and 90% EtOH for 10 minutes each, infiltrated in 90% EtOH:LR white resin at 1:1 (20 minutes), 1:2 (40 minutes), and pure LR white, 60 minutes and overnight. They were embedded in LR white and polymerized in a vacuum oven at 50 °C for 2 days. For regular electron microscopy, tissues were further fixed in 2.5% glutaraldehyde-0.1 M cacodylate buffer overnight at 4 °C and postfixed in osmium tetroxide, en bloc stained in 2% uranyl acetate in 50% EtOH, dehydrated in 70%, 80%, 95% EtOH, and propylene oxide, infiltrated and embedded in Epon 812.

For post-embedding immunogold labeling, thin sections of LR white-embedded tissues were collected on Formvar-coated nickel grids and incubated with the following tau antibodies, MC1 (P. Davis, Albert Einstein College of Medicine, New York, NY, USA), Tau12 (L. Binder, Northwestern Univ., Chicago, IL, USA), followed by respective secondary antibodies conjugated to 18-nm colloidal gold particle (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Sections were stained with uranyl acetate and lead citrate before examination with a Philips 208S electron microscope (FEI, Hillsboro, OR, USA), fitted with a bottom-mounted Gatan 831 Orius digital camera (Gatan, Pleasanton, CA, USA). Digital images were processed using adobe photoshop CS5 (64 bit) software.

2.6. Immunohistochemical staining

Immunohistochemistry was performed on brain tissue from rTg4510 and nonTg mice at various ages (1.5 to 8-month-old rTg4510 and non-transgenic mice; $n = 14$ and $n = 7$, respectively). Formalin fixed brains were paraffin embedded and cut into sagittal ($5\ \mu\text{m}$) sections. Immunohistochemistry was performed with the Dako Universal Autostainer (Dako, Carpinteria, CA, USA). Primary antibodies used mouse monoclonal IgG1 antibody MC1 (1:1000), which recognized tau conformation with a compact folding state (Jicha et al., 1997). Counter staining with hematoxylin was performed on representative sections to align sections across experimental animals. Images were taken by ScanScope XT digital scanner (Aperio, Vista, CA, USA) to digitize each microscope slide. A quantitative analysis of tau burden was performed using ImageScope version 10 software (Aperio), unbiased computer-assisted image analysis program. We used a positive pixel count algorithm that measures the percent positivity of diaminobenzidine (DAB) staining in a selected region. The cortex ($6.0\text{--}12.8\ \text{mm}^2$ per section) and hippocampus ($1.2\text{--}3.5\ \text{mm}^2$ per section) were traced and analyzed with each staining.

3. Results

3.1. Immunohistochemical detection of tau burden in hippocampus and cortex

Immunohistochemical staining for MC1, an antibody recognizing a pathologic confirmation of tau was included to show the progression of tau pathology in rTg4510 mice (Fig. 1). Dense staining for MC1 occurs at around the age of 6 months in cortex and hippocampus in rTg4510 mice (Fig. 1A). However, at the age of 1.5–2.5 months there is already an observable increase in tau burden in rTg4510 mice compared with nonTg in cortex (Fig. 1B) and hippocampus (Fig. 1C). In spite of this evidence of early staining, at the light microscopic level there is no noticeable staining for MC1 in corpus callosum of rTg4510 mice until the age of 6 months (Fig. 1D).

3.2. Diffusion tensor imaging measures

We examined DT-MRI anisotropy indices of the brains of rTg4510 and nonTg mice of different ages (2.5-, 4.5-, and 8-month-old). Resulting FA maps are shown in Fig. 2. Compared with 8-month-old nonTg mice, rTg4510 mice show an age-related reduction in FA contrast in WM (CC, gCC). This is not observed in 2.5-month-old rTg4510 mice (Fig. 2). In addition, we find a reduced overall brain size in 8-month-old rTg4510 mice as previously reported with other imaging modalities (Perez et al., 2013; Yang et al., 2011). Fig. 3 summarizes the results for FA values across various GM and WM regions. In general, GM regions showed FA values near 0.2 as previously reported (Boska et al., 2007) and is indicative of low anisotropy (low directionality of diffusion within GM). WM regions showed FA values ranging from 0.3 to close to 0.6 (Boska et al., 2007), indicative of greater anisotropy than GM. There were no significant differences between regional values for FA in nonTg mice of different ages. rTg4510 showed a significant age-related decline in FA values, which was mostly observed in WM structures at 8 months. This effect was observed in anterior commissure (2-way ANOVA main effect $p < 0.05$; strain: $F_{1, 23} = 12.8$, $p = 0.002$; age: $F_{2, 23} = 4.6$, $p = 0.02$), CC (strain: $F_{1, 23} = 24.6$, $p < 0.0001$; age: $F_{2, 23} = 11.6$, $p = 0.0003$), IC (strain: $F_{1, 23} = 9.4$, $p = 0.005$; age: $F_{2, 23} = 5.3$, $p = 0.01$), sCC (strain: $F_{1, 23} =$

4.2, $p = 0.05$; age: $F_{2, 23} = 5.4$, $p = 0.01$), and Fmb (strain: $F_{1, 23} = 10.9$, $p = 0.003$; age: $F_{2, 23} = 1.8$, $p = 0.2$). The only non-WM structure showing this effect was the SN (strain: $F_{1, 23} = 2.0$, $p = 0.2$; age: $F_{2, 23} = 9.3$, $p = 0.001$); however, it is expected that WM from the IC may have been jointly sampled within this ROI (Fig. 3). Red, green, blue encoded FA maps showed an age-related reduction in organized WM and a “thinning” of WM of the CC, which is most pronounced when comparing 8MO nonTg with 8MO rTg4510 mice (Fig. 3). D_R , D_{Ax} , and D_{ave} values are summarized in Supplementary Table 1. Overall, there was a lack of effect of strain and age on D_{Ax} and D_{ave} and an increased D_R . The latter effect was observed to be significant in CC, sCC, and Fmb (2-way ANOVA Sidak multiple comparison test, $p < 0.05$).

Douaud et al. (2011) recently reported a novel diffusion tensor imaging (DTI) metric in AD (mode or A_{MO}) that describes the geometric characteristics of FA (Ennis and Kindlmann, 2006). We included A_{MO} in our analyses and the results are shown in Fig. 4. Most of the WM and GM regions of 2.5 and 4.5-month-old mice (both nonTg and rTg4510) show A_{MO} values ranging from +0.5 to close to +1. This excluded the CC in 2.5MO rTg4510 mice, which showed values between 0 and -0.25 (approaching a more planarlike diffusion ellipsoid). Indeed, rTg4510 mice of all ages showed low A_{MO} values in gCC than nonTg mice (2 ANOVA main effect for strain: $F_{1, 23} = 7.9$, $p = 0.009$; Fig. 4). rTg4510 mice showed a significant change in A_{MO} values in 3 regions, one region (CTX) showing an increase in A_{MO} and the remaining 2 regions showing a decrease. This effect was observed in CTX (strain: $F_{1, 23} = 0.2$, $p = 0.6$; age: $F_{2, 23} = 5.5$, $p = 0.01$), SN (strain: $F_{1, 23} = 9.9$, $p = 0.0008$; age: $F_{2, 23} = 5.2$, $p = 0.03$), and sCC (strain: $F_{1, 23} = 11.1$, $p = 0.0004$; age: $F_{2, 23} = 6.7$, $p = 0.02$). The observed changes in A_{MO} were not observed in nonTg mice (Fig. 4).

To summarize the DTI findings, our results indicate that rTg4510 expressing human P301L tau show an age-related reduction in FA, with increased D_R , reduced A_{MO} and unchanged D_{ave} and D_{Ax} . Most of the observed reductions in FA were in WM areas and a consistent strain related effect was observed in corpus callosum.

3.3. Ultrastructural observations

In nonTg mice, corpus callosum contained tightly packed bundles of axons and unmyelinated processes, even at the age of 14 months (Fig. 5A). In contrast, in rTg4510 mice many axons were variably swollen with degenerated debris, and were widely separated (Fig. 5B). In addition, many oligodendrocytes showed large filamentous aggregates, and glial fibrils of reactive astrocytes permeated the space. By IEM, aggregates of tau-positive filaments were detected in axons and swollen unmyelinated processes in rTg4510 mice at the age of 4 months and 12 months (Fig. 5C and D).

4. Discussion

The present data show an age-related decline in FA of various WM structures in a mouse model of tauopathy. A combined assessment of various anisotropy indicators strongly suggest a reduced directionality of WM tissue diffusivity associated with increased radial diffusivity (and no difference axial diffusivity values) in rTg4510 mice compared with nonTg. This is consistent with the observed reduction in anisotropic diffusion that is

reported in several DT-MRI studies of AD (Amlie et al., 2013; Bozzali et al., 2001; Medina et al., 2006; Selnes et al., 2013; Serra et al., 2010; Stenset et al., 2011). The description of FA through the estimation of the mode of anisotropy provided a marker for early detection of tau related pathology in the rTg4510 mouse. The corpus callosum of rTg4510 mice showed a significantly reduced mode of anisotropy compared with controls, which is similar to what has been reported previously in AD subjects and has been discussed within the context of a “disorganization” of WM tracts (Douaud et al., 2011). Alterations in the preferential orientation of bulk diffusion as a consequence of reductions in WM tracts of a particular direction could underlie the observed reduction in mode. Our IEM data provide initial support for our DTI findings. Further studies are needed to confirm and explore various potential mechanisms contributing to increased radial diffusivity, reduced FA, and alterations in the tensor mode shown in Fig. 4. At the age of 4 months, rTg4510 mice show tau inclusions in an axon. We also observed unmyelinated processes. However, there is a possibility that the unmyelinated processes correspond to dendrites present within the corpus callosum (Fig. 5). At the age of 12 months we observed novel inclusions in myelin sheath, as well as axons and unmyelinated processes. Because immunogold staining was used this ensures that these are not confused with neurofilament staining. Interestingly, at the age of 14 months we also observed a “disorganized” pattern of myelinated fiber arrangement with enlarged inter-axonal spaces. These were not observed in age-matched nonTg mice. The results are not conclusive but encourage further investigations of interactions between tau associated pathologic changes and WM integrity at the ultrastructural level. The literature does support a general reduction in WM (myelinated projections), increased astrogliosis (Brun and Englund, 1986; Shin et al., 1992), which is hypothesized here to underlie part of the long-term effects in 8-month-old rTg4510 mice. At this later age there is a significant decline in the estimated mode of anisotropy in the splenium. Conversely, the sampled frontal cortex area showed an increase in mode that might reflect loss of fibers of a particular orientation within the GM tissue. The fact that the nonTg mice (serving as controls) showed high consistency in the estimated anisotropy indices in the present study is noteworthy and in part supports the validity of the present methods, and the findings. These data point specifically to overexpressed mutant P301L tau as a contributor to the observed differences in DTI metrics between nonTg and rTg4510 mice.

The presence of WM pathology in AD is well documented (Braak and Braak, 1996; Brun and Englund, 1986; Kowall and Kosik, 1987; Shin et al., 1992; Umahara et al., 2002). However, it is not until recently that noninvasive diffusion imaging techniques have become available to study the progression of WM pathology and age-related changes in WM and GM in AD, MCI, and normal aging. The bulk of the DT-MRI studies are in human subjects with a minor portion of studies carried out with ex vivo or live transgenic mice. In general, reductions in FA and increased D_{ave} have been reported across several studies. There is less agreement on the specific WM regions but parallels can be drawn on the cortical locations of anisotropy changes and the associated WM tracts. Reduced FA and increased D_{ave} were reported in AD patients compared with age- and sex-matched healthy volunteers in the corpus callosum as well as in temporal, frontal, and parietal lobes (Bozzali et al., 2001). A study including MCI and AD subjects compared with healthy volunteers reported a reduced FA (Medina et al., 2006). According to the authors this was mostly observed in posterior

WM regions although the individually reported areas appeared more widespread across the cingulate, frontal, and parietal areas (Medina et al., 2006). More recently, DT-MRI studies have incorporated longitudinal follow-up designs, cognitive, and functional assessments in MCI and AD subjects, as well as CSF measurements of diagnostic biomarkers such as total tau, phosphorylated tau, $A\beta_{1-42}$. In a short 3-month follow-up study, MCI and AD patients demonstrated reduced FA compared with baseline and compared with healthy subjects, which was observed in fornix, splenium, and anterior portions of cingulum bundle in AD, but only in splenium for MCI (Mielke et al., 2009). A 3-year follow up study examined changes in WM integrity in MCI subjects that were either stable and did not progress into AD, or had probable AD having progressed to AD no earlier than 2 years after a baseline DT-MRI scanning (Douaud et al., 2013). This group observed that MCI with probable AD had lower subregional volumes in hippocampus, amygdala, and temporal pole than stable MCI subjects. Using tract-based spatial statistics and correcting for multiple comparisons the authors did not find significant changes in FA, or D_{ave} between these categories of MCI subjects. However, A_{MO} (mode of anisotropy) was significantly lower in the left fimbria (Douaud et al., 2013). Overall, most of the above DT-MRI studies confirm the presence of WM pathology accompanying volumetric and cellular changes in GM areas and these also confirm the in vivo progression of pathology in WM even at stages of MCI preceding AD proper, and in healthy aging.

In spite of the notable progress in the validation of DT-MRI as a diagnostic and predictive tool when used in combination with other biological and cognitive assessments in human subjects, direct causal relations with specific AD pathologies is needed. This is perhaps an attainable goal through the use of DT-MRI studies in transgenic mouse models of AD. This was a major impetus for the present work. The rTg4510 mouse shows AD-related tau pathology in an age-progressive manner (Santacruz et al., 2005). Moreover, the controlled regional expression of P301L tau is set by the Ca^{2+} -calmodulin kinase II promoter and this maintains the early presence of mutant tau within forebrain structures, thereby modeling a fundamental feature of FTDP-17-Tau (Goedert, 2004). rTg4510 mice over-express a pathogenic species of human P301L tau (with 4 microtubule binding pockets and absence of an N-terminal segment, 4R0N) that aggregates in paired helical filaments in neuronal soma, axon, synaptic terminals, and that progresses into intracellular NFTs (Ramsden et al., 2005). Destabilization of microtubules (Barten et al., 2012) and axonal swellings containing vesicular structures filled with axonal debris has been reported (Lin et al., 2005). Hyperphosphorylated tau accumulation in cortex and hippocampus appears as early as 2.5 months (Ramsden et al., 2005), consistent with the present results for A_{MO} in cortex of 2.5-month-old mice. The presence of NFTs (Ramsden et al., 2005) and alterations in synaptic firing and the biophysical properties of neurons (Crimins et al., 2012) may predate the cognitive behavioral deficits observed at 4–6 months (Ramsden et al., 2005). Thus, the tangles themselves do not explain the present results. Whether or not under naturally occurring circumstances tau expression leads to, or somehow facilitates or just adds upon, these changes in AD and FTDP-17-Tau is not yet known (Ballatore et al., 2007). However, tau overexpression has been associated with a loss of myelinated axons, and/or their surrounding oligodendritic cells (Bartzokis, 2011), which also accumulate tau (Ren et al., 2013; Shin et al., 1992). The cause of this is also in need of further investigation, but it is

likely that early stages of AD and MCI might occur alongside, or even be predated by, a WM-linked mechanism that could include tau aggregates as one of its central components (Bartzokis, 2011). Localized WM inflammation as a consequence of microglial activation and migration to the injury site (in the case of axonal pathology) may in part underlie the A_{MO} results. It is particularly important that we observe this effect in the corpus callosum, which mimics findings in human AD (Douaud et al., 2011). Long-range frontal cortical myelinated axon projections transit through this region of interest and may be a target for further investigation in future work. At the latest stage in 8-month-old rTg4510 mice we observed that the cortex has an increased A_{MO} , which could reflect a loss of axons transiting through GM. Detailed anatomic analysis following in vivo imaging is warranted to gain further details regarding the present results.

Consistent with the aforementioned human DT-MRI studies, we find that rTg4510 mice show an age-related decline in WM integrity. In our present work, the mouse model used expresses P301L tau in forebrain regions including the hippocampal formation, temporal lobe, frontal cortex, and amygdala (Ramsden et al., 2005; Santacruz et al., 2005), which are areas reported to show reduced WM FA in DT-MRI studies of AD and MCI (Bozzali et al., 2001; Douaud et al., 2011; Selnes et al., 2013). Axonal transport of tau across these brain areas might be feasible and/or their accumulation in major tracts connecting these (de Calignon et al., 2012) (Fig. 5). Within axons, tau-related destabilization of microtubules, which are essential for retro- and anterograde transport mechanisms, may be an integral part of axonal and neuronal loss (Barten et al., 2012). Also, increased microgliosis near myelinated axons, breakdown of myelin, and axonal loss may underlie reduced FA because of a loss of directionality of diffusion and/or increased barriers for diffusion under these conditions. In support of this contention, a recent magnetic resonance spectroscopy study in 5MO rTg4510 mice revealed greater levels of chemical markers for gliosis compared with nonTg mice (Yang et al., 2011). Increased microglial cells within the damaged site may lead to reduced anisotropy. Conversely, overall loss of WM projections along multiple orientations could potentially underlie reduced FA. This could in part explain the reduced increased D_R and reduced A_{MO} in some WM structures. However, as with DT-MRI of AD, the reason for this decline in WM FA (and reduced A_{MO}) and increased D_R in rTg4510 mice at this time remains unclear.

There are several methodological aspects of DTI that should be mentioned in light of the present interpretations. As with many other techniques there are drawbacks that should receive attention. These include confounds produced by head motion, susceptibility-induced artifacts, low signal to noise and its effects on quantitative parameters, perfusion effects on the DTI metrics, and partial volume effects. In many respects the implemented experimental conditions may have averted some of these pitfalls. Data were collected using an EPI sequence to minimize the effects of motion on diffusion-sensitized image quality. Experiments were carried out at a high field, and this is the first DTI study carried out at the current field strength in mice, which provided the needed high signal to noise. Although, $B = 0$ images were collected and this might lead to sensitivity to perfusion-induced artifacts, we collected 6 $B = 0$ images with an additional 42 noncollinear sampling directions which provide satisfactory conditions for diffusion parameter estimates. Localized manual adjustments of higher order shims and the EPI gradient delays, along with collection of

phase images to correct for N/2 ghosting and image distortions all improved the quality of the collected diffusion weighted images. As for motion artifacts, it is important to note that mice were well restrained and affine registration of diffusion weighted images to the first $B = 0$ image improved the image quality further. Differences in brain size may also have an impact on the final quantitative diffusion measures. As we have noted in previous work (Perez et al., 2013; Santacruz et al., 2005), rTg4510 mice show reduced total brain size and this could make the sampling of WM and gray matter structures difficult for ROIs below an effective voxel size. Higher spatial resolution, with significant sacrificing of acquisition time is perhaps the best solution for this issue.

Additional experimental caveats should also be considered for improving future work. We estimated diffusion shape measures according to Westin (O'Donnell and Westin, 2011; Westin et al., 2002) (data not shown). These include spherical, planar, and linear shape measures similar to those described by the reported tensor mode. While the tensor mode in the cortex of aged rTg4510 mice was increased to over 0.3, there was no overt increase of linear shape measures or decrease of spherical shape measures in the analysis according to Westin model. This might be discrepant and according to the Ennis and Kindlmann article (2006) the tensor mode can also be influenced by low signal to noise conditions, which is likely for the present cortical measurements. However, it is important to keep in mind that while FA has been correlated with the tensor mode (Ennis and Kindlmann, 2006), Westin linear shape measures have not. Regardless, the mode values in the cortex should be cautiously interpreted given the impact of low signal to noise in this region on this metric, which may not be the case for WM.

Another point of consideration is that the altered mode of anisotropy in the corpus callosum was already noticeable in rTg4510 mice at the age of 2–3 months (Fig. 3). An alternative explanation to the one favored here is that this may indicate abnormalities of postnatal development of the mutant strain rather than age-related neurodegeneration because of overexpressed tau. Increased value of mode of anisotropy in the cortex of 8 to 11-month-old rTg4510 mice might arise from delayed onset of postnatal developmental deficits. Effects of perinatal and early postnatal expression of mutant tau could be circumvented by induced suppression of transgene expression with doxycycline over these periods to examine whether rTg4510 mice recapitulate white matter pathologies in elderly patients. This will be confirmed in future work. Finally, no regular (transmission) electron microscopic images of younger mouse brains were collected, and it accordingly remains undetermined whether “disorganization” of axonal and myelin morphologies is noticeable at the age of 2.5–4 months thereby inducing changes in DT-MRI parameters.

To our knowledge this is the first in vivo DT-MRI study of a mouse model of tauopathy. It is interesting to note that in one in vivo DT-MRI study of 14 months APP/PS1 mice there was an increased FA or D_{ave} , or both, in cingulum, corpus callosum, internal capsule, and other WM and GM structures (Qin et al., 2013), which contradicts the present findings in the P301L tau mouse. However, another recent ex vivo DT-MRI experiment in mice containing the Swedish double mutation of APP showed reduced relative anisotropy in the corpus callosum at 12 months (Harms et al., 2006). Although the mice used in the present study were significantly younger (oldest 10.8 months in the 8 months group), these express

significant levels of tau with significant neuronal loss at this age. The reason for this discrepancy is not clear but may lie in underlying differences in the role of each of these pathologic proteins (APP, PS1, and tau) in myelin breakdown and/or axonal damage and repair processes in each of these strains of mice (APP/PS1 and P301L mice) (Bartzokis, 2011). Improvements in the current methods and the examination of DT-MRI quantities in mice with other well-known pathologies related to AD and other neurodegenerative diseases should help provide some added insight into the interpretations of DT-MRI data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2013.12.009>.

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Fig. 1.

Progression of tau pathology in rTg4510 mice. (A) Light microscopic images of sagittal brain sections from 1.5-, 2.5-, 4-, 6-, 8-month-old (1.5 M, 2.5 M, 4 M, 6 M, and 8 M) rTg4510 mice. The sections were immunostained with MC1 antibody. The counterstain (blue) was hematoxylin. Bar = 2 mm. (B and C) Quantitative analysis of percent MC1-positive burden of each age in cerebral cortex (B) and hippocampus (C). Values are means $\pm$ standard error. (D) Representative images of corpus callosum region from 1.5-, 2.5-, 4-, 6-, 8-month-old (1.5 M, 2.5 M, 4 M, 6 M, and 8 M) rTg4510 mice. Bar = 20 μ m.

Fig. 2. Fractional anisotropy (FA) maps of ~2.5-month-old, ~4.5-month-old, 8-month-old rTg4510 mice, and an 8-month-old nonTg mouse as a control. (A) Selected ROIs are highlighted in atlas maps of the mouse brain shown on the left column (Paxinos). ROI abbreviations: anterior commissure (AC), corpus callosum (CC), genu (gCC), splenium (sCC), striatum (STR), frontal cortex (CTX), hippocampus (HPC), fimbria (Fmb), amygdala (Amy), internal capsule (IC), substantia nigra (SN). (B) FA maps in nonTg and rTg4510 mice at 2.5, 4.5, and 8-month-old. Abbreviations: nonTg, nontransgenic; ROIs, regions of interest.

Fig. 3. (A) Mean (μ standard error) FA values of various ROIs of nonTg and rTg4510 mice at ~2.5-month-old, ~4.5-month-old, and 8-month-old mice. Graphs highlight age-dependent changes in FA for several gray and white matter regions. * Indicates significantly different from 2.5-month-old rTg4510 mice, +significant difference from corresponding age-matched nonTg group, and ϕ significantly different from 4.5-month-old rTg4510, ($p < 0.05$, 2 way ANOVA with Tukey multiple comparison test). (B) Color-encoded FA maps of ~2.5-month-old, ~4.5-month-old, 8-month-old rTg4510 mice, and an 8-month-old nonTg mouse as a control. Arrows indicate direction of anisotropy (R-horizontal, G-vertical, B-out from page). Abbreviations: FA, fractional anisotropy; nonTg, nontransgenic; ROIs, regions of interest.

Fig. 4.

Mode of anisotropy (A_{MO}) of ~2.5-month-old, ~4.5-month-old, 8-month-old nonTg and age-matched rTg4510 mice. (A) Ellipsoid shapes shown overlying the bar graphs illustrate the relation with values of A_{MO} ; however, these are not based on actual modeling. A_{MO} ranges from -1 for a planar or “pancake-like” (oblate) diffusion ellipsoid shape to a value of $+1$ for a linear or “cigar-like” (prolate) ellipsoid shape. (B) Note the negative values for corpus callosum and its subregions (genu and splenium) as early as 2.5 months in rTg4510 compared with nonTg mice. (C) Age-related changes in FA for several ROIs. Large asterisk indicates group main effect for strain (rTg4510 versus nonTg, but not age). All data presented as mean $\pm$ standard error. *Indicates significantly different from 2.5-month-old rTg4510 mice, +significant difference from corresponding age-matched nonTg group, and ϕ significantly different from 4.5-month-old rTg4510, ($p < 0.05$, 2-way ANOVA with Tukey multiple comparison test). Abbreviations: ANOVA, analysis of variance; FA, fractional anisotropy; nonTg, nontransgenic; ROIs, regions of interest.

Fig. 5.

(A) Electron micrograph of corpus callosum in 14-month-old nonTg mouse showing tightly packed myelinated axons and unmyelinated neuritic processes. Bar = 0.5 μ m. (B) In 14-month-old Tg mouse, axons containing various degenerated debris were swollen and widely separated. An oligodendrocyte (Olig) has large cytoplasmic inclusion (*). Note the presence of fibrillary bundle of reactive astrocyte (Ast). Bar = 1.0 μ m. (C) Immunogold labeling of tau (MC1) in an unmyelinated process (arrowhead) in 4-month-old rTg4510 mouse. Bar = 0.5 μ m. Inset enlargement shows labeled filaments (arrows point to gold particles) in dense cytoplasmic matrix. Inset bar = 0.2 μ m. (D) Immunogold labeling of tau (Tau12) in an unmyelinated process (arrow) and an axon (arrowhead) in 12-month-old rTg4510 mouse. Bar = 0.5 μ m. Insets enlargements show labeled filaments in pale cytoplasm (arrows point to gold particles). Inset bars = 0.2 μ m.