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Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology

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Abstract

Alzheimer's disease (AD) is a progressively debilitating brain disorder pathologically defined by extracellular amyloid plaques, intraneuronal neurofibrillary tangles, and synaptic disintegrity. AD has not been widely considered a disease of white matter, but more recent evidence suggests the existence of abnormalities in myelination patterns and myelin attrition in AD-afflicted human brains. Herein, we demonstrate that triple-transgenic AD (3xTg-AD) mice, which harbor the human amyloid precursor protein Swedish mutant transgene, presenilin knock-in mutation, and tau P301L mutant transgene, exhibit significant region-specific alterations in myelination patterns and in oligodendrocyte marker expression profiles at time points preceding the appearance of amyloid and tau pathology. These immunohistochemical signatures are coincident with age-related alterations in axonal and myelin sheath ultrastructure as visualized by comparative electron microscopic examination of 3xTg-AD and non-transgenic mouse brain tissue. Overall, these findings indicate 3xTg-AD mice represent a viable model in which to examine mechanisms underlying AD-related myelination and neural transmission defects that occur early during pre-symptomatic stages of the disease process.

Keywords

Myelin; Oligodendrocyte; Myelin basic protein; CNPase; 3xTg-AD; Electron microscopy

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that leads to severe memory loss and cognitive impairment. Current estimates suggest that AD afflicts more than 5 million individuals in the United States and 24 million people worldwide, with this number

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projected to double in 20 years (Qiu, et al. 2007). Pathologically, AD is characterized by the temporal and spatial progression of amyloid plaques arising from deposition of the fibrillogenic amyloid-beta (A β) peptide and other proteins, and neurofibrillary tangles due to the hyperphosphorylation of the microtubule-stabilizing protein tau. At the cellular level, AD is further associated with decreased synaptic density and eventual neuronal loss within brain regions underlying cognition and memory formation (DeKosky and Scheff 1990).

AD is classically considered a disease of gray matter; however, appreciable aberrations in white matter (WM) have also been reported (Firbank, et al. 2007a; Roher, et al. 2002). WM tracts within the cerebral cortex and subcortical regions, which are comprised of myelinated white fibers, provide connectivity that is central to cognition, emotion, and consciousness (Filley 2005; Makris, et al. 1997). Myelination occurs in a “heterochronological” pattern, in which different brain regions myelinate via disparate timelines. Studies of affected areas in the AD brain, including the hippocampus and entorhinal cortex, suggest that the regions most vulnerable to AD pathogenesis exhibit the most protracted course of myelination (Bartzokis 2004; Bartzokis, et al. 2004; Duyckaerts 2004). Quantitative volumetric magnetic resonance imaging (MRI) assessments have revealed WM atrophy within these regions in brains of incipient and mildly afflicted AD patients (Bobinski, et al. 1999; de Toledo-Morrell, et al. 2000a; de Toledo-Morrell, et al. 2000b; Dickerson, et al. 2001; Insausti, et al. 1998).

Cells comprising the oligodendocyte lineage mediate the process of myelination, and disease-related insults may detrimentally impact the ability of these cells to properly myelinate axonal processes in vulnerable brain regions, thereby leaving them more susceptible to stressors such as oligomeric A β peptides, neuroinflammatory molecules, and reactive oxygen species (ROS) (Roher, et al. 2002). We posit that alterations in myelin occur prior to overt AD pathology in the earliest affected brain regions as a result of AD-associated assault on the oligodendrocyte cell lineage. This process results in disrupted transmission of neuronal impulses leading to memory loss and alterations in axonal integrity.

Herein, we utilize the triple transgenic mouse model (3xTg-AD), developed in Dr. Frank LaFerla's laboratory, which harbors three mutations: human presenilin-1 M146V (PS1^{M146V}), human amyloid precursor protein Swedish mutation (APP^{Swe}), and the P301L mutation of human tau (tau^{P301L}) (Oddo, et al. 2003b). The 3xTg-AD mouse develops amyloid plaques and neurofibrillary tangle pathology in a temporal and spatial progression that mimics the pathogenic stages observed in humans, and thus serves as a valuable tool to study pathophysiological mechanisms associated with AD and the vetting of newly designed disease-modifying therapeutics (Oddo, et al. 2003a; Oddo, et al. 2006). At 6 months of age, 3xTg-AD mice exhibit a profound defect in long-term potentiation (LTP) and paired-pulse facilitation, while the earliest cognitive impairment in 3xTg-AD mice manifests at 4 months of age as a diminution in long-term spatial memory retention (Billings, et al. 2005; Oddo, et al. 2003b). These prior observations suggest that dysfunctional neural transmission resulting from disrupted action potentials and myelin integrity along the axon contribute to the electrophysiologic and behavioral deficits in these mice. To that end, we examined the myelination status of 3xTg-AD and non-transgenic mice at various ages preceding the appearance of overt amyloid and tau-related pathologies using immunohistochemical, biochemical, and ultra-structural analyses. We find that 3xTg-AD mice exhibit striking aberrations in oligodendrocyte marker expression and myelin ultrastructure as compared to age-matched control mice in regions of the brain shown to be earliest affected in AD, implying that myelination processes represent vulnerable targets that contribute to the regional and temporal nature of disease progression.

Materials and Methods

Mouse strains

Triple-transgenic Alzheimer's disease (3xTg-AD) mice were created previously (Oddo, et al. 2003a; Oddo, et al. 2003b). Age-matched male mice were used for all experiments (n=4 per experimental group for immunocytochemical studies, luxol fast blue staining, and biochemical assays, n=3 per experimental group for electron microscopy). Age-matched male 129/C57BL/6 mice were employed as non-transgenic (Non-Tg) controls. All animal housing and procedures were performed in compliance with guidelines established by the University of Rochester.

Electron microscopy

Mice were perfused transcardially, brains removed, sectioned to 1 μ m coronal sections to include the hippocampal formation at 1.70 mm to 3.40 mm posterior to Bregma, and placed in fresh fixative (4.0% paraformaldehyde/2.0% glutaraldehyde in 0.1M sodium cacodylate buffer). Sections were further trimmed to include the Schaffer collaterals, post-fixed in 1.0% osmium tetroxide, dehydrated, and embedded in Epon. Ultra-thin sections were counterstained with uranyl acetate followed by lead citrate and examined using a Hitachi 7100 transmission electron microscope. Images for myelin sheath morphology/integrity were captured using a MegaView III digital camera and AnalySIS (Soft Imaging Systems, Lakewood, Colorado) software. Images were captured at 10,000X and 30,000X magnification.

Preparation of brain tissue homogenates and Western blot analyses

Frozen tissues from the hippocampal region of the brain were homogenized in lysis buffer (50mM Tris-HCl pH 7.5, 5mM EDTA, 1% Triton X-100) with protease inhibitors (Sigma, St. Louis, MO). Samples were centrifuged (15,000 rpm at 4°C for 15 min.), supernatants collected, and samples assayed for protein concentration using the DC protein assay (Bio-Rad, Hercules, CA). Protein lysates (100 μ g) were electrophoretically separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to polyvinylidene difluoride membrane (Bio-Rad). The following primary antibodies were used: 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), myelin basic protein (MBP; Millipore, Billerica, MA; 1:1000), or MAG (Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1:1000) followed by the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody incubation. Blots were developed using enhanced chemiluminescence (Perkin Elmer, Waltham, MA).

Immunohistochemical detection of MBP, CNPase, and neurofilament

Brains from age-matched non-transgenic control and 3xTg-AD mice were sectioned coronally (30 μ m) and stored in cryoprotectant at -20°C until processed for immunohistochemistry, or were paraffin-embedded and sectioned at 7 μ m onto slides using a cryostat where noted. Antigen retrieval was performed as previously described (Peterson, et al. 2007). Tissues were permeabilized using 0.4% Triton-X100 in phosphate buffer (PB), blocked with 10% goat serum in PB and incubated with the primary antibody for CNPase or MBP (Chemicon International, 1:1000 and 1:200, respectively). Sections were subsequently rinsed in PB and incubated with Alexa Fluor 568 or 488 goat anti-mouse and goat anti-rat secondary antibody (Molecular Probes, 1:2000). Additional sections were incubated with a neurofilament-specific antibody, 2H3, (Developmental Studies Hybridoma Bank, 1:500) following permeabilization in 0.1% TX-100 instead of 0.4% TX-100, while PBS was used throughout instead of PB. Stained sections were visualized using the Leica SP1 (Leica Microsystems Inc, Bannockburn, IL) microscope and images captured under 40X magnification. Three consecutive sections from each mouse (4 mice total per genotype per age) for the different regions of the hippocampus

and entorhinal cortex were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD).

Luxol fast blue stain for myelin

Luxol fast blue staining was performed on 7- μ m paraffin-embedded sections as previously described (Wu, et al. 2008). Stained sections were visualized using an Olympus AX-70 microscope equipped with a motorized stage (Olympus, Melville, NY) and images were captured under 40X magnification.

Immunohistochemical analysis of Na_v1.6

Sections for Na_v1.6 staining were first deparaffinized, washed in PB, incubated with 3% H₂O₂ (Sigma) to quench endogenous peroxidase activity, permeabilized using 0.4% TX-100 in PB, blocked using 5% normal goat serum in 0.4% Triton-X 100 in PB and then incubated with mouse monoclonal anti-Na_v1.6 (Antibodies Inc., Davis, CA; 1:1000) overnight in blocking solution. Slides were rinsed with PB and incubated with biotinylated goat anti-mouse immunoglobulin (Vector Laboratories, Burlingame, CA). Sections were washed with PB, the HRP activity conjugated, and the antigen-antibody complexes were subsequently developed using a DAB peroxidase kit according to manufacturer's instructions for nickel enhancement (Vector Laboratories). Stained sections were analyzed using an Olympus DP71 microscope equipped with a motorized stage (Olympus, Melville, NY) and images were captured under 100X magnification.

Statistical Analysis

Statistical analysis was performed by means of 2-way ANOVA followed by the Bonferroni post-test using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA).

Results

Axonal myelin sheath integrity is compromised in 3xTg-AD mice

Previously, imaging studies and biochemical analyses have revealed white matter damage in the brains of human AD patients and significant decreases in levels of the myelin and oligodendrocyte proteins, myelin basic protein (MBP) and 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) (Roher, et al. 2002; Vlkolinsky, et al. 2001). MBP is a major constituent of CNS myelin and mature oligodendrocytes, whereas CNPase is expressed throughout the oligodendrocyte cell lineage (Baumann and Pham-Dinh 2001). Several studies have also revealed that the entorhinal cortex and hippocampus are amongst the brain regions that are affected during the earliest stages in AD (Braak and Braak 1991; Braak and Braak 1997; de Toledo-Morrell, et al. 2004; Pennanen, et al. 2004). In the present study, we utilized the 3xTg-AD mouse model, given its staged development of AD-related amyloid and tau pathologies (Oddo, et al. 2003b), to examine patterns of potential white matter degeneration and myelin/oligodendrocyte protein expression as it may relate to the spatial evolution of previously described AD pathologies in this model.

We initially examined myelin integrity at the ultrastructural level in the 3xTg-AD mice by means of electron microscopy. The assessment was performed on fibers of the Schaffer collateral pathway, which project from the CA3 to the CA1 region of the hippocampus. Gross deterioration of axonal morphology in 3xTg-AD mice at both 2 and 6 months of age compared to age-matched non-transgenic (Non-Tg) control mice was apparent (Figure 1A-E). Further examination of myelin sheath organization demonstrated splitting of the major denseline, accompanied by accumulation of dense bodies, which indicates age-related changes in myelin

integrity (Figure 1F-J) (Hinman and Abraham 2007). Quantitation of percent of axonal and myelin sheath alterations in each field revealed significant signs of disruption in 3xTg-AD mice at both 2 and 6 months of age compared to non-transgenic mice (Figure 1E and J). Changes were also observed in 6 month-old Non-Tg mice apparently due to normative aging, but these alterations were significantly enhanced in 3xTg-AD mice.

Temporal profiling of myelin and oligodendrocyte marker expression by Western blotting

To provide an overall view of myelin/oligodendrocyte marker expression, we assessed MBP and CNPase steady-state levels in microdissected hippocampus and entorhinal cortex tissue samples of age-matched 3xTg-AD and Non-Tg mice using Western blotting. We posited that global diminution of MBP and/or CNPase steady-state profiles in 3xTg-AD mice could underlie the myelin disruptions observed by electron microscopy in these animals. We readily detected CNPase as a single band migrating at 46 kDa, while the 14 kDa, 17.24/17.22 kDa, and 18.5 kDa isoforms of the MBP protein were detected in mouse brain tissue homogenates (Figure 2A and B). Densitometry was performed on resultant X-ray films and levels of each protein of interest were normalized to the β -actin internal control signal. Although not reaching statistical significance for any of the intra and inter-group comparisons, the relative steady-state global levels of CNPase and MBP trended toward increases in the hippocampus of 2 month-old 3xTg-AD versus Non-Tg mice, and decreases in 6-month old 3xTg-AD versus age-matched Non-Tg mice (Figure 2C and E). CNPase expression in the entorhinal cortex revealed a non-statistically significant decline in both 2 and 6 month-old 3xTg-AD versus Non-Tg mice (Figure 2D), whereas MBP expression appeared to decline at 2 months but increase in 6 month-old 3xTg-AD mice as compared to age-matched Non-Tg control mice (Figure 2F). While these Western blot data are indicative of MBP and CNPase expression throughout the entire hippocampus and entorhinal cortex regions, we were unable to discern sub-regional differences of expression that might be prevalent in 3xTg-AD mice, as such differences would be diluted out by the contribution of unaffected regions. Given this possibility, we further assessed sub-regional protein expression patterns of CNPase and MBP and myelin integrity in age-matched 3xTg-AD and Non-Tg mice using immunohistochemistry.

Age-related changes in myelin and oligodendrocyte marker status in sub-regions of the hippocampus and entorhinal cortex

Given the heterogeneous nature of myelination in the hippocampus and entorhinal cortex, we chose to assess the CA1, dentate gyrus, and CA3 regions of the hippocampus and the superficial layers II/III, as well as deep layers IV/V, of the entorhinal cortex region. Examination of the CA1 hippocampal region revealed marked decreases in both MBP (green) and CNPase (red) staining in 2 and 6 month-old 3xTg-AD mice compared to age-matched controls (Figure 3A-H). No significant changes were observed in the dentate gyrus of 3xTg-AD mice compared to Non-Tg mice at both 2 and 6 months of age (Figure 3S and T). Similarly, no significant changes were observed in the CA3 regions of 3xTg-AD mice compared to Non-Tg mice at both ages (data not shown). Entorhinal cortex assessments (Figure 4) revealed no significant age-related difference in CNPase and MBP staining intensity in layers IV/V (Figure 4S and T), while significant changes were observed between the cohorts at 6 months of age in the superficial layers II/III of the entorhinal cortex (Figure 4Q and R). Collectively, these results indicate that myelin and oligodendrocyte marker expression profiles are compromised in the CA1 hippocampal region and superficial layer II/III of the entorhinal cortex region of the 3xTg-AD mice. Of note, the axons arising from entorhinal cortex layers III principally form the perforant pathway and have direct input to the CA1 region of the hippocampus (van Groen, et al. 2003). These results further support our findings that appreciable sub-region-specific myelin disruption is present in young 3xTg-AD mice.

Axonal staining patterns in hippocampal and cortical structures of 3xTg-AD and Non-Tg mice are comparable

It is possible that the diminished sub-region intensity of CNPase and MBP staining in 6 month-old 3xTg-AD mice might reflect decreased axonal density that arises from altered development and/or age-dependent neurotoxicities imparted by transgenically expressed human APP^{Swe}/Tau^{P301L} proteins in these genetically engineered mice. To globally assess axonal density patterns, we performed immunohistochemistry for mouse neurofilament protein, 2H3, on coronal sections obtained from age-matched Non-Tg control and 3xTg-AD mice.

Neurofilaments are type IV family of intermediate filaments found in high concentrations along the axons of vertebrate neurons and serve as major elements of the cytoskeleton supporting the axonal cytoplasm (Chou, et al. 2001). Qualitative analyses revealed comparable levels of neurofilament staining in the CA1 hippocampal region of 2 and 6 month-old 3xTg-AD mice compared to age-matched non-transgenic control mice (Figure 5A-D). Immunocytochemical staining of the entorhinal cortex region demonstrated qualitatively similar levels of 2H3 protein compared to age-matched control mice (Figure 5E-H). Thus, it appeared that the altered oligodendrocytes/myelin protein staining patterns in 6 month-old 3xTg-AD mice were not due to an overt compromise in axonal densities in the hippocampus or entorhinal cortex.

Temporal disruption of myelination in the brains of 3xTg-AD mice

To further demonstrate myelination defects in the CA1 hippocampal region and layer II/III of entorhinal cortex of 3xTg-AD mice, we performed luxol fast blue staining for myelin on paraffin-embedded coronal sections. There was a qualitative increase in fiber density in the CA1 region of the Non-Tg control mice at 6 months of age compared to 2 month-old mice, as depicted by an increase in blue staining intensity (Figure 6C and A, respectively). Conversely, a reduction in myelin fiber density was observed in 6 month-old compared to 2 month-old 3xTg-AD mice (Figure 6D and B, respectively). The overall staining intensity in 6 month-old 3xTg-AD mice was markedly decreased as compared to age-matched Non-Tg control mice. Patterns of luxol fast blue staining in the entorhinal cortex were similar to those in the hippocampus at each of the time points for both 3xTg-AD and Non-Tg mice, where 6 month-old 3xTg-AD mice exhibited diminished staining of myelinated fibers (Figure 6E-H).

Na_v1.6 channel expression correlates with levels of myelination in 3xTg-AD and Non-Tg control mice

During embryonic development, Na_v1.6 channels replace Na_v1.2 channels at the nodes of Ranvier as myelination progresses and nodes mature and are abundantly expressed in the adult CNS (Boiko, et al. 2001; Rios, et al. 2003). The Na_v1.6 channels are important for impulse propagation through high frequency firing at the nodes of Ranvier (Zhou and Goldin 2004). To this end, we used Na_v1.6 channel expression as detected via immunohistochemistry as an indication of functional integrity of axons and synaptic transmission in the 3xTg-AD mice. CA1 hippocampus analyses revealed reduced Na_v1.6 channel staining in 6 month-old 3xTg-AD mice compared to age-matched Non-Tg mice (Figure 7D). No apparent changes in staining intensities were detectable between 2 and 6 month-old 3xTg-AD mice, while staining appeared more intense in 6 month-old Non-Tg mice. Similar expression patterns were observed in the entorhinal cortex region of the brain with a noticeable qualitative decline in channel staining in 6 month-old 3xTg-AD mice compared to age matched Non-Tg control mice (Figure 7H). These data suggest that the alterations in myelin integrity in 3xTg-AD mice may impart deleterious effects on axonal impulse propagation and may underlie the previously described disturbances in electrophysiological functioning at these early ages (LaFerla and Oddo 2005).

Discussion

The progressive evolution of memory deficits in human AD patients strongly correlate with pathological events related to synaptic dysfunction, and to a lesser extent with presentation of classical amyloid plaque and neurofibrillary tangle formation (Dickson, et al. 1995; Masliah, et al. 2001; Teipel, et al. 2007; Terry, et al. 1991). Preservation of axonal integrity, which is maintained by an enveloping myelin sheath, is critical for facilitating normal synaptic function and neural transmission. Magnetic resonance diffusion-weighted imaging studies have correlated cognitive decline to axonopathy in AD-afflicted individuals (Chen, et al. 2007; Deppe, et al. 2007; Firbank, et al. 2007a; Firbank, et al. 2007b; Huang and Auchus 2007; Mayzel-Oreg, et al. 2007; Ringman, et al. 2007; Stahl, et al. 2007), while numerous post-mortem studies have provided evidence of white matter disruption in human AD brains (Braak and Braak 1996; Hyman, et al. 1986; Roher, et al. 2002), which may be indicative of altered connectivity and white matter dysfunction. These observations strongly implicate the oligodendrocyte as a key cell type that may be detrimentally affected early in the disease process. Lending support to this hypothesis, myelin-producing oligodendrocytes have also been shown to be highly susceptible to A β peptide exposure (Xu, et al. 2001; Zeng, et al. 2005), inflammatory signaling and oxidative stress byproducts (Arnett, et al. 2001; Balabanov, et al. 2007; Cammer and Zhang 1999; Deng, et al. 2004; Gu, et al. 1999; Haynes, et al. 2003; McCracken, et al. 2000; Roth, et al. 2005; Zeng, et al. 2005), all of which are intimately associated with human AD.

Several studies assessing disruption of WM in asymptomatic individuals at risk for inherited familial AD and patients with mild cognitive impairment syndrome have been conducted (Bartzokis, et al. 2006; Parente, et al. 2008; Ringman, et al. 2007). Parente et al. employed diffusion tensor imaging (DTI) to assess WM tracts and observed significantly lower fractional anisotropy in MCI and AD patients compared to control patients (Parente, et al. 2008). Bartzokis and colleagues correlated age-related slowing in cognitive processing speed and myelin breakdown in later-myelinating white matter of asymptomatic “younger-old” individuals at an increased risk for AD attributed to Apolipoprotein E genotype (Bartzokis, et al. 2007). Previous research has demonstrated a decrease in brain cholesterol levels and oligodendrocyte/myelin proteins, MBP and CNPase, in human AD patients (Roher, et al. 2002). Similarly, our observations indicate that myelination is disrupted at earliest pathological stages in sub-regions of the entorhinal cortex and hippocampus of 3xTg-AD mice. We also find a decline in steady-state MBP and CNPase expression levels in the CA1 hippocampal region and superficial layers of the entorhinal cortex of 3xTg-AD mice, whereas other sub-regions did not reveal significant modulations in marker expression. The perforant pathway, a principal cortical input to the hippocampus, constitutes fibers principally arising from the superficial layer III of the entorhinal cortex to the CA1 hippocampal region (van Groen, et al. 2003). The perforant pathway is important in spatial learning and previous evidence indicates cognitive impairment of 3xTg-AD mice at 6 months of age when subjected to the Morris water maze behavioral test (Billings, et al. 2005). Hyman et al. previously reported pathological changes in the cells of origin of the perforant pathway in AD-afflicted individuals (Hyman, et al. 1986). More importantly, Ringman and colleagues have demonstrated a decline in the area of perforant pathway bilaterally and mean whole-brain WM fractional anisotropy using DTI in preclinical and even presymptomatic familial AD mutation carriers (Ringman, et al. 2007).

The Schaffer collaterals project from the CA3 region of the hippocampus to the CA1 region represent another important pathway of the hippocampus. The Schaffer collateral axons have been the subject of previously published long-term potentiation measurements and synaptic response assessments in 3xTg-AD mice (Oddo, et al. 2003b). We found a significant deterioration of axonal ultrastructure comprising this pathway in 6 month-old 3xTg-AD mice. Furthermore, evidence of major denseline myelin sheath splitting was apparent, along with the

accumulation of cytoplasmic dense bodies. Previous studies have shown similar white matter granular dense bodies to be ubiquitin-immunopositive and abnormal ubiquitination of white matter axons has been observed in brains from patients with multiple sclerosis (Giordana, et al. 2002; Hinman and Abraham 2007). Therefore, it is plausible that the observed aggregation of dense bodies in the 3xTg-AD mice is indicative of ubiquitin-positive conjugates. Of note, we have performed immunohistochemistry on brain tissue from 3xTg-AD and Non-Tg control mice to detect ubiquitin-positive conjugates. The overall intensity of ubiquitin staining is significantly higher in the 3xTg-AD mice as compared to Non-Tg control animals at 6 months of age. Moreover, there appear to be regional differences in ubiquitin staining and the majority of the ubiquitin-positive conjugates appear to be associated with what appear to be neurons (Desai and Bowers, unpublished observations).

In humans with AD-related dementia, memory dysfunction is linked to synaptic loss and neuritic dystrophy (Grace and Busciglio 2003). However, changes in axonal transmission as a result of disrupted myelination have not been unequivocally dismissed in these cases. Na_v1.6 channels, a major isoform of sodium channels, are localized to myelinated zones and are critical for impulse propagation at the nodes of Ranvier (Boiko, et al. 2001; Zhou and Goldin 2004). We observed altered levels of Na_v1.6 channel immunopositivity in 3xTg-AD mice concordant with declining myelin status. Multiple sclerosis studies have demonstrated intense Na_v1.6 channel immunoreactivity at nodes of Ranvier in normal appearing white matter, whereas demyelinated axons exhibit less intense immunoreactivity that appears in a patchy rather than diffuse distribution (Black, et al. 2007; Craner, et al. 2004; Wittmack, et al. 2005). It is possible that downregulation of Na_v1.6 channel expression in the CA1 hippocampal region and entorhinal cortex of 3xTg-AD mice is indicative of modulated channel function, which could significantly impact axonal conduction and synaptic transmission.

Our study is not the first to describe myelination defects in the setting of a mouse model of AD. Harms et al. employed DTI to assess white matter integrity in APP^{swe} mice and observed a significant decrease in the area of corpus collosum (CC) at 12 and 15 months of age, a time when amyloid plaque deposition is readily detectable (Harms, et al. 2006). Others assessed axonopathy in the spinal cords of APP/PS1 double-transgenic mice using ultrastructural analyses and found evidence of thinned and detached myelin leaflets that correlated with indications of intraneuronal A β accumulation and extracellular plaque deposition (Wirhth, et al. 2007; Wirhth, et al. 2006). However, these authors did not present ultrastructural data relating to brain myelination defects. Using DTI, Song et al. assessed white matter in outbred PDAPP mice, another amyloidogenic model of AD, at 3 months of age (pre-amyloid pathology) and at later ages when extensive pathology was already resident (>12 months). The authors observed that WM tracts in PDAPP mice exhibited altered relative anisotropy values at 13 months of age, but not at 2 months of age when compared to non-transgenic control mice (Song, et al. 2004). The electron microscopy data from Song and colleagues suggested that there were dystrophic axons proximal to amyloid plaques, which had partially or completely lost myelin sheathing within the white matter. Given these previous observations, we believe that our present study is the first to report ultrastructural evidence of myelination defects in a mouse model of AD at ages that precede overt amyloid or tau pathology.

In summary, we demonstrate that myelination processes are vulnerable targets, which when compromised, may contribute to the regional and temporal nature of early disease progression of AD. Elucidating the molecular trigger(s) of this defect as it relates to AD-related transgene expression in the 3xTg-AD mouse model could provide valuable insight into how subtle white matter alterations in human AD arise. Moreover, acquisition of such information will likely uncover novel therapeutic targets.

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References

- Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci* 2001;4(11):1116–22. [PubMed: 11600888]
- Balabanov R, Strand K, Goswami R, McMahon E, Begolka W, Miller SD, Popko B. Interferon-gamma-oligodendrocyte interactions in the regulation of experimental autoimmune encephalomyelitis. *J Neurosci* 2007;27(8):2013–24. [PubMed: 17314297]
- Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. *Neurobiol Aging* 2004;25(1):5–18. [PubMed: 14675724]author reply 49–62
- Bartzokis G, Lu PH, Geschwind DH, Edwards N, Mintz J, Cummings JL. Apolipoprotein E genotype and age-related myelin breakdown in healthy individuals: implications for cognitive decline and dementia. *Arch Gen Psychiatry* 2006;63(1):63–72. [PubMed: 16389198]
- Bartzokis G, Lu PH, Geschwind DH, Tingus K, Huang D, Mendez MF, Edwards N, Mintz J. Apolipoprotein E affects both myelin breakdown and cognition: implications for age-related trajectories of decline into dementia. *Biol Psychiatry* 2007;62(12):1380–7. [PubMed: 17659264]
- Bartzokis G, Lu PH, Mintz J. Quantifying age-related myelin breakdown with MRI: novel therapeutic targets for preventing cognitive decline and Alzheimer's disease. *J Alzheimers Dis* 2004;6(6 Suppl):S53–9. [PubMed: 15665415]
- Baumann N, Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 2001;81(2):871–927. [PubMed: 11274346]
- Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron* 2005;45(5):675–88. [PubMed: 15748844]
- Black JA, Newcombe J, Trapp BD, Waxman SG. Sodium channel expression within chronic multiple sclerosis plaques. *J Neuropathol Exp Neurol* 2007;66(9):828–37. [PubMed: 17805013]
- Bobinski M, de Leon MJ, Convit A, De Santi S, Wegiel J, Tarshish CY, Saint Louis LA, Wisniewski HM. MRI of entorhinal cortex in mild Alzheimer's disease. *Lancet* 1999;353(9146):38–40. [PubMed: 10023955]
- Boiko T, Rasband MN, Levinson SR, Caldwell JH, Mandel G, Trimmer JS, Matthews G. Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. *Neuron* 2001;30(1):91–104. [PubMed: 11343647]
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)* 1991;82(4):239–59. [PubMed: 1759558]
- Braak H, Braak E. Evolution of the neuropathology of Alzheimer's disease. *Acta Neurol Scand Suppl* 1996;165:3–12. [PubMed: 8740983]
- Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging* 1997;18(4):351–7. [PubMed: 9330961]
- Cammer W, Zhang H. Maturation of oligodendrocytes is more sensitive to TNF alpha than is survival of precursors and immature oligodendrocytes. *J Neuroimmunol* 1999;97(1–2):37–42. [PubMed: 10408976]
- Chen SQ, Kang Z, Hu XQ, Hu B, Zou Y. Diffusion tensor imaging of the brain in patients with Alzheimer's disease and cerebrovascular lesions. *J Zhejiang Univ Sci B* 2007;8(4):242–7. [PubMed: 17444598]
- Chou YH, Helfand BT, Goldman RD. New horizons in cytoskeletal dynamics: transport of intermediate filaments along microtubule tracks. *Curr Opin Cell Biol* 2001;13(1):106–9. [PubMed: 11163141]

- Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG. Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na⁺/Ca²⁺ + exchanger. *Proc Natl Acad Sci U S A* 2004;101(21):8168–73. [PubMed: 15148385]
- de Toledo-Morrell L, Dickerson B, Sullivan MP, Spanovic C, Wilson R, Bennett DA. Hemispheric differences in hippocampal volume predict verbal and spatial memory performance in patients with Alzheimer's disease. *Hippocampus* 2000a;10(2):136–42. [PubMed: 10791835]
- de Toledo-Morrell L, Goncharova I, Dickerson B, Wilson RS, Bennett DA. From healthy aging to early Alzheimer's disease: in vivo detection of entorhinal cortex atrophy. *Ann N Y Acad Sci* 2000b; 911:240–53. [PubMed: 10911878]
- de Toledo-Morrell L, Stoub TR, Bulgakova M, Wilson RS, Bennett DA, Leurgans S, Wu J, Turner DA. MRI-derived entorhinal volume is a good predictor of conversion from MCI to AD. *Neurobiol Aging* 2004;25(9):1197–203. [PubMed: 15312965]
- DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol* 1990;27(5):457–64. [PubMed: 2360787]
- Deng W, Wang H, Rosenberg PA, Volpe JJ, Jensen FE. Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. *Proc Natl Acad Sci U S A* 2004;101(20):7751–6. [PubMed: 15136737]
- Deppe M, Duning T, Mohammadi S, Schwindt W, Kugel H, Knecht S, Ringelstein EB. Diffusion-tensor imaging at 3 T: detection of white matter alterations in neurological patients on the basis of normal values. *Invest Radiol* 2007;42(6):338–45. [PubMed: 17507803]
- Dickerson BC, Goncharova I, Sullivan MP, Forchetti C, Wilson RS, Bennett DA, Beckett LA, deToledo-Morrell L. MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease. *Neurobiol Aging* 2001;22(5):747–54. [PubMed: 11705634]
- Dickson DW, Crystal HA, Bevana C, Honer W, Vincent I, Davies P. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol Aging* 1995;16(3):285–98. [PubMed: 7566338]discussion 298–304
- Duyckaerts C. Looking for the link between plaques and tangles. *Neurobiol Aging* 2004;25(6):735–9. [PubMed: 15165696]discussion 743–6
- Filley CM. Neurobehavioral aspects of cerebral white matter disorders. *Psychiatr Clin North Am* 2005;28(3):685–700. 697–8. [PubMed: 16122574]
- Firbank MJ, Blamire AM, Krishnan MS, Teodorczuk A, English P, Gholkar A, Harrison R, O'Brien JT. Atrophy is associated with posterior cingulate white matter disruption in dementia with Lewy bodies and Alzheimer's disease. *Neuroimage* 2007a;36(1):1–7. [PubMed: 17412610]
- Firbank MJ, Blamire AM, Krishnan MS, Teodorczuk A, English P, Gholkar A, Harrison RM, O'Brien JT. Diffusion tensor imaging in dementia with Lewy bodies and Alzheimer's disease. *Psychiatry Res* 2007b;155(2):135–45. [PubMed: 17408930]
- Giordana MT, Richiardi P, Trevisan E, Boghi A, Palmucci L. Abnormal ubiquitination of axons in normally myelinated white matter in multiple sclerosis brain. *Neuropathol Appl Neurobiol* 2002;28(1):35–41. [PubMed: 11849561]
- Grace EA, Busciglio J. Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy. *J Neurosci* 2003;23(2):493–502. [PubMed: 12533609]
- Gu C, Casaccia-Bonnel P, Srinivasan A, Chao MV. Oligodendrocyte apoptosis mediated by caspase activation. *J Neurosci* 1999;19(8):3043–9. [PubMed: 10191321]
- Harms MP, Kotyk JJ, Merchant KM. Evaluation of white matter integrity in ex vivo brains of amyloid plaque-bearing APPsw transgenic mice using magnetic resonance diffusion tensor imaging. *Exp Neurol* 2006;199(2):408–15. [PubMed: 16483571]
- Haynes RL, Folkert RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, Kinney HC. Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. *J Neuropathol Exp Neurol* 2003;62(5):441–50. [PubMed: 12769184]
- Hinman JD, Abraham CR. What's behind the decline? The role of white matter in brain aging. *Neurochem Res* 2007;32(12):2023–31. [PubMed: 17447140]
- Huang J, Auchus AP. Diffusion tensor imaging of normal appearing white matter and its correlation with cognitive functioning in mild cognitive impairment and Alzheimer's disease. *Ann N Y Acad Sci* 2007;1097:259–64. [PubMed: 17413027]

- Hyman BT, Van Hoesen GW, Kromer LJ, Damasio AR. Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann Neurol* 1986;20(4):472–81. [PubMed: 3789663]
- Insausti R, Juottonen K, Soininen H, Insausti AM, Partanen K, Vainio P, Laakso MP, Pitkanen A. MR volumetric analysis of the human entorhinal, perirhinal, and temporopolar cortices. *AJNR Am J Neuroradiol* 1998;19(4):659–71. [PubMed: 9576651]
- LaFerla FM, Oddo S. Alzheimer's disease: Abeta, tau and synaptic dysfunction. *Trends Mol Med* 2005;11(4):170–6. [PubMed: 15823755]
- Makris N, Worth AJ, Sorensen AG, Papadimitriou GM, Wu O, Reese TG, Wedeen VJ, Davis TL, Stakes JW, Caviness VS, others. Morphometry of in vivo human white matter association pathways with diffusion-weighted magnetic resonance imaging. *Ann Neurol* 1997;42(6):951–62. [PubMed: 9403488]
- Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW Jr. Morris JC. Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. *Neurology* 2001;56(1):127–9. [PubMed: 11148253]
- Mayzel-Oreg O, Assaf Y, Gigi A, Ben-Bashat D, Verchovsky R, Mordohovitch M, Graif M, Hendler T, Korczyn A, Cohen Y. High b-value diffusion imaging of dementia: application to vascular dementia and alzheimer disease. *J Neurol Sci* 2007;257(1–2):105–13. [PubMed: 17360001]
- McCracken E, Valeriani V, Simpson C, Jover T, McCulloch J, Dewar D. The lipid peroxidation by-product 4-hydroxynonenal is toxic to axons and oligodendrocytes. *J Cereb Blood Flow Metab* 2000;20(11):1529–36. [PubMed: 11083227]
- Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging* 2003a;24(8):1063–70. [PubMed: 14643377]
- Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 2003b;39(3):409–21. [PubMed: 12895417]
- Oddo S, Caccamo A, Tran L, Lambert MP, Glabe CG, Klein WL, LaFerla FM. Temporal profile of amyloid-beta (Abeta) oligomerization in an in vivo model of Alzheimer disease. A link between Abeta and tau pathology. *J Biol Chem* 2006;281(3):1599–604. [PubMed: 16282321]
- Parente DB, Gasparetto EL, da Cruz LC Jr. Domingues RC, Baptista AC, Carvalho AC. Potential role of diffusion tensor MRI in the differential diagnosis of mild cognitive impairment and Alzheimer's disease. *AJR Am J Roentgenol* 2008;190(5):1369–74. [PubMed: 18430857]
- Pennanen C, Kivipelto M, Tuomainen S, Hartikainen P, Hanninen T, Laakso MP, Hallikainen M, Vanhanen M, Nissinen A, Helkala EL, others. Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. *Neurobiol Aging* 2004;25(3):303–10. [PubMed: 15123355]
- Peterson EB, Mastrangelo MA, Federoff HJ, Bowers WJ. Neuronal Specificity of HSV/Sleeping Beauty Amplicon Transduction In Utero Is Driven Primarily by Tropism and Cell Type Composition. *Mol Ther.* 2007
- Qiu C, De Ronchi D, Fratiglioni L. The epidemiology of the dementias: an update. *Curr Opin Psychiatry* 2007;20(4):380–5. [PubMed: 17551353]
- Ringman JM, O'Neill J, Geschwind D, Medina L, Apostolova LG, Rodriguez Y, Schaffer B, Varpetian A, Tseng B, Ortiz F, others. Diffusion tensor imaging in preclinical and presymptomatic carriers of familial Alzheimer's disease mutations. *Brain* 2007;130(Pt 7):1767–76. [PubMed: 17522104]
- Rios JC, Rubin M, St Martin M, Downey RT, Einheber S, Rosenbluth J, Levinson SR, Bhat M, Salzer JL. Paranodal interactions regulate expression of sodium channel subtypes and provide a diffusion barrier for the node of Ranvier. *J Neurosci* 2003;23(18):7001–11. [PubMed: 12904461]
- Roher AE, Weiss N, Kokjohn TA, Kuo YM, Kalback W, Anthony J, Watson D, Luehrs DC, Sue L, Walker D, others. Increased A beta peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. *Biochemistry* 2002;41(37):11080–90. [PubMed: 12220172]
- Roth AD, Ramirez G, Alarcon R, Von Bernhardi R. Oligodendrocytes damage in Alzheimer's disease: beta amyloid toxicity and inflammation. *Biol Res* 2005;38(4):381–7. [PubMed: 16579521]

- Song SK, Kim JH, Lin SJ, Brendza RP, Holtzman DM. Diffusion tensor imaging detects age-dependent white matter changes in a transgenic mouse model with amyloid deposition. *Neurobiol Dis* 2004;15(3):640–7. [PubMed: 15056472]
- Stahl R, Dietrich O, Teipel SJ, Hampel H, Reiser MF, Schoenberg SO. White matter damage in Alzheimer disease and mild cognitive impairment: assessment with diffusion-tensor MR imaging and parallel imaging techniques. *Radiology* 2007;243(2):483–92. [PubMed: 17456872]
- Teipel SJ, Stahl R, Dietrich O, Schoenberg SO, Perneczky R, Bokde AL, Reiser MF, Moller HJ, Hampel H. Multivariate network analysis of fiber tract integrity in Alzheimer's disease. *Neuroimage* 2007;34(3):985–95. [PubMed: 17166745]
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 1991;30(4):572–80. [PubMed: 1789684]
- van Groen T, Miettinen P, Kadish I. The entorhinal cortex of the mouse: organization of the projection to the hippocampal formation. *Hippocampus* 2003;13(1):133–49. [PubMed: 12625464]
- Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. *Neurobiol Aging* 2001;22(4):547–53. [PubMed: 11445254]
- Wirths O, Weis J, Kayed R, Saido TC, Bayer TA. Age-dependent axonal degeneration in an Alzheimer mouse model. *Neurobiol Aging* 2007;28(11):1689–99. [PubMed: 16963164]
- Wirths O, Weis J, Szczygielski J, Multhaup G, Bayer TA. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol* 2006;111(4):312–9. [PubMed: 16520967]
- Wittmack EK, Rush AM, Hudmon A, Waxman SG, Dib-Hajj SD. Voltage-gated sodium channel Nav1.6 is modulated by p38 mitogen-activated protein kinase. *J Neurosci* 2005;25(28):6621–30. [PubMed: 16014723]
- Wu QZ, Yang Q, Cate HS, Kemper D, Binder M, Wang HX, Fang K, Quick MJ, Marriott M, Kilpatrick TJ, others. MRI identification of the rostral-caudal pattern of pathology within the corpus callosum in the cuprizone mouse model. *J Magn Reson Imaging* 2008;27(3):446–53. [PubMed: 17968901]
- Xu J, Chen S, Ahmed SH, Chen H, Ku G, Goldberg MP, Hsu CY. Amyloid-beta peptides are cytotoxic to oligodendrocytes. *J Neurosci* 2001;21(1):RC118. [PubMed: 11150354]
- Zeng C, Lee JT, Chen H, Chen S, Hsu CY, Xu J. Amyloid-beta peptide enhances tumor necrosis factor- α -induced iNOS through neutral sphingomyelinase/ceramide pathway in oligodendrocytes. *J Neurochem* 2005;94(3):703–12. [PubMed: 16033420]
- Zhou W, Goldin AL. Use-dependent potentiation of the Nav1.6 sodium channel. *Biophys J* 2004;87(6):3862–72. [PubMed: 15465873]

Figure 1. Electron microscopy reveals distorted axons and granulation in myelin sheaths in the hippocampus of 3xTg-AD mice

Ultrastructural analyses of axonal and myelin integrity were carried out on the Schaffer collateral pathway. Morphological axonal abnormalities and intact axonal structures (A-D) are represented for 2 and 6 month-old Non-Tg and 3xTg-AD mice. * represents abnormal structure and # represents intact axonal structure. Myelin sheath disruptions with dense granular inclusions (F-I) are also illustrated. Arrowheads point to disrupted myelin sheaths with granular inclusions. Quantitative analyses of altered axons (E) and granulation in myelin (J) are significant in the 3xTg-AD mice. Scale bar in Panel D indicates 2000 nm and Panel I indicates 500 nm. Error bars indicate standard deviation. “***” indicates $p < 0.001$ and “*” indicates $p < 0.05$.

Figure 2. Assessment of pan-oligodendrocyte and myelin protein marker expression in the brains of 3xTg-AD and Non-Tg mice

Representative Western blots for CNPase, MBP, and β -actin in the hippocampus of 2 and 6 month-old Non-Tg and 3xTg-AD mice (A) and the entorhinal cortex region (B) are shown. Semi-quantitative analyses of western blots for CNPase in the hippocampus (C) and entorhinal cortex (D) region of Non-Tg and 3xTg-AD mice are illustrated. Similarly, analyses of MBP in the hippocampus (E) and entorhinal cortex (F) region of the mice are also shown. C = non-transgenic control mice, Tg = 3xTg-AD mice, MW = molecular weight.

Figure 3. Sub-regional expression differences of CNPase and MBP in the hippocampus of 3xTg-AD mice

Immunohistochemical detection of CNPase and MBP in the CA1 region (A-D and E-H, respectively) and dentate gyrus region (I-L and M-P, respectively) of the hippocampus in 2 and 6 month-old Non-Tg and 3xTg-AD mice is illustrated. Quantification of CNPase immunofluorescence in the CA1 (Q) and dentate gyrus (S) region of 2 and 6 month-old Non-Tg and 3xTg-AD mice and MBP immunofluorescence in the CA1 (R) and dentate gyrus (T) region of these mice are also demonstrated. The scale bars depict 50 μ m. Error bars indicate standard deviation. “**” indicates $p < 0.01$, “***” indicates $p < 0.001$.

Figure 4. Alterations in CNPase and MBP expression in the sub-regions of the entorhinal cortex of 3xTg-AD mice

Representative immunohistochemical images for CNPase and MBP for the superficial layers II/III (A-D and E-H, respectively) and deep layers IV/V (I-L and M-P, respectively) are illustrated for coronal brain sections from 2 and 6 month-old Non-Tg and 3xTg-AD mice. CNPase immunofluorescence quantification for layer II/III (Q) and layer IV/V (S) for 2 and 6 month-old mice is shown. Similar analyses of MBP immunofluorescence for layer II/III (R) and layer IV/V (T) are also illustrated. The scale bars depict 50 μ m. Error bars indicate standard deviation. “***” indicates $p < 0.001$.

Figure 5. Axon integrity appears normal in 3xTg-AD mice at both 2 and 6 months of age
 Qualitative immunohistochemical detection of neurofilament protein 2H3, is illustrated for the CA1 region (A-D) of the hippocampus and layer II/III (E-H) of the entorhinal cortex in 2 and 6 month-old Non-Tg and 3xTg-AD mice. The scale bars depict 50 μ m.

Figure 6. Myelination is disrupted at 6 months of age in the brains of 3xTg-AD mice
 Luxol fast blue staining for the CA1 hippocampus region (A-D) for 2 and 6 month-old Non-Tg and 3xTg-AD mice is illustrated. Arrows point to myelinated processes. Images were also captured for layer II/III (E-H) of the entorhinal cortex region of Non-Tg and 3xTg-AD mice as indicated. The scale bars depict 50 μm .

Figure 7. Impaired $\text{Na}_v1.6$ channel expression in 3xTg-AD mice at 6 months of age
 $\text{Na}_v1.6$ channel expression in the CA1 region (A-D) of the hippocampus and layer II/III (E-H) of the entorhinal cortex region in 2 and 6 month-old Non-Tg and 3xTg-AD mice is shown. Arrows point to representative axons expressing $\text{Na}_v1.6$ channels along the processes. Neuronal cell bodies in the entorhinal cortex are labeled with an "N". The scale bars depict 5 μm .