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Diffusion MRI Detects Longitudinal White Matter Changes in the 3xTg-AD Mouse Model of Alzheimer's Disease

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Keywords

Diffusion MRI; Diffusional Kurtosis Imaging; 3xTg-AD mouse; white matter; Alzheimer's disease

1. Introduction

Diffusion MRI (dMRI) is widely used as a noninvasive means of detecting changes in brain tissue microstructure associated with both pathology and aging [1]. Its sensitivity to microstructure stems from its ability to quantify the motion of water molecules on length scales commensurate with the size of neurons and glia.

For Alzheimer's disease (AD), dMRI holds promise as a method of monitoring disease progression that is potentially useful in assessing the efficacy of drug therapies. To better understand the meaning of alterations of diffusion parameters in the context of AD, dMRI

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experiments with transgenic mouse models of AD pathology are valuable in allowing for controlled studies from the earliest stages of disease.

Several different transgenic mouse models have contributed substantially to our understanding of AD pathogenesis [2,3]. Among them is the triple transgenic AD mouse model (3xTg-AD) that progressively develops amyloid and tau-related pathologies with a temporal and regional-specific profile comparable to the human AD [4,5]. This model has been applied extensively to investigate AD [6–11], including for a few prior imaging studies [12–15]. 3xTg-AD mice show intracellular accumulation of amyloid beta ($A\beta$) at 3 months of age, with extracellular plaques appearing in the neocortex and hippocampus at 6 months of age. Tau pathology begins at 6 months, when phosphorylated tau becomes detectable in the hippocampus, and develops into neurofibrillary tangles after 12 months of age [4, 5, 16]. Interestingly, the first detectable pathological features in this model are alterations in overall myelination patterns leading to white matter (WM) disruption. Changes in oligodendrocyte and myelin marker expression are noticeable in the hippocampus and entorhinal cortex as early as 2 months of age [17–19] and precede the first cognitive impairments and $A\beta$ plaques [17].

Despite these well characterized morphological and histochemical WM matter abnormalities, only one prior dMRI study has investigated WM in the 3xTg-AD mouse model [14]. Employing the specific dMRI method of diffusion tensor imaging (DTI), this study reported no WM differences in older (11–17 months) 3xTg-AD mice relative to controls.

Diffusional kurtosis imaging (DKI) is an alternative dMRI technique that extends DTI by quantifying the non-Gaussian behavior of water diffusion, contributing additional information beyond what is given by DTI [20,21]. Besides the diffusion indices conventionally obtained with DTI, DKI also provides measures of diffusional non-Gaussianity, such as mean (MK), axial ($K_{||}$) and radial ($K_{\perp}$) kurtoses. These extra parameters can help to characterize both normal and abnormal brain tissue cytoarchitecture. DKI has been used in human studies to investigate tissue microstructural changes associated with aging [22–25] and AD [26,27], particularly for assessing WM abnormalities [28–31]. Furthermore, the extra diffusional kurtosis (DK) measures have a demonstrated sensitivity to brain pathology in a variety animal models [32–34], including AD mouse models [35,36].

Thus, the goal of this study was to investigate the sensitivity of DKI to WM microstructural changes associated with age and different stages of disease progression in the 3xTg-AD mouse model.

2. Methods

2.1 AD mouse model

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Medical University of South Carolina (MUSC) and conducted in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Four female 3xTg-AD mice were studied longitudinally at 4.5, 9, 12, 15 and 18 months of

age. The 3xTg-AD mice possess three mutations of human presenilin-1 M146V, human amyloid precursor protein Swedish mutation, the P301L mutation of human tau, and develop both A β and neurofibrillary tangles (NFTs) in a temporal and spatial pattern that is similar to human AD pathology. All mice were housed in temperature- and humidity-controlled rooms on a 12-h light/dark cycle (lights on at 6:00 AM) in an accredited animal care facility.

2.2 MRI acquisition

Mice were anesthetized using an isoflurane vaporizer set at the following percentages: 3% for induction, 2% during pilot scanning, and 1.5% during data acquisition. An animal monitoring unit (SA instruments, Inc., model 1025, Stony Brook, NY) was used to record respiration and rectal temperature. Respiration was measured with a pressure transducer placed under the abdomen just below the ribcage. Body temperature was maintained using ventilated warm air, controlled by a feedback circuit between the heater and thermistor. After induction, mice were placed on a mouse holder and restrained using a mouse tooth bar (Bruker, T10146) and ear bars (Bruker, T10147) placed in the auditory canal. Compressed air was used as the carrier gas and delivered at a flow rate (1L/min) to a nose cone positioned around the tooth bar, where gases mixed with air and passed over the rodent's nose. All animals were maintained at 37.0 ± 0.2 °C and respiration ranged between 70 and 80 breaths per minute during scanning.

The *in vivo* MRI experiments were performed on a 7T BioSpec 70/30 horizontal scanner (Bruker BioSpin, Ettlingen, Germany) running Paravision 5.1 acquisition software, and equipped with a 12 cm inner diameter actively shielded gradient system (440 mT/m) with a quadrature volume coil (T128038) for signal transmission and a mouse brain array coil (T11765) for signal reception. A 2-shot spin-echo echo planar imaging (SE-EPI) sequence was used for DKI acquisition. Sequence parameters were: TR/TE=3750/32.6ms, $\delta/\delta' = 5/18$ ms, slice thickness=0.7 mm, 15 coronal slices with no gap (Figure 1), data matrix=128×128, image resolution=156×156 μm^2 , 2 repetitions, 10 b-value=0 images, followed by 30 diffusion encoding gradient directions with 4 b-values for each gradient direction (0.5, 1, 1.5, 2 ms/ μm^2), and fat suppression using a frequency selective RF pulse with a flip angle of 105° degrees to minimize chemical shift artifacts. Total acquisition time=33 minutes.

2.3 DKI post-processing and Image analysis

DKI post-processing was performed using DKE [37] (<http://nitrc.org/projects/dke>). Parametric maps were obtained by fitting dMRI signal measurements to the DKI signal model for each voxel using a linearly constrained weighted linear least squares fitting algorithm, which generated the diffusion and kurtosis tensors. These two tensors were then used to calculate parametric maps for all diffusion measures [20,38]. In our analysis, we considered the four diffusion tensor (DT) parameters of mean diffusivity (MD), axial diffusivity ($D_{\parallel}$), radial diffusivity ($D_{\perp}$), and fractional anisotropy (FA). MD corresponds to the diffusivity averaged over all diffusion directions, $D_{\parallel}$ corresponds to the diffusivity in the direction of the principal diffusion tensor eigenvector, $D_{\perp}$ corresponds to the diffusivity averaged over all diffusion directions perpendicular to the principal diffusion tensor eigenvector, and FA quantifies the anisotropy of the diffusion tensor. We similarly

considered the four DK parameters of mean kurtosis (MK), axial kurtosis ($K_{\parallel}$), radial kurtosis ($K_{\perp}$), and kurtosis fractional anisotropy (KFA). These are kurtosis analogs of the DT metrics that quantify diffusional non-Gaussianity and provide complementary information about the diffusion dynamics [20,38,39]. All 4 b-values for each gradient direction (0.5, 1, 1.5, 2 ms/ μm^2) were used for calculating all the diffusion parameters. It is worth noting that, due to the inclusion of non-Gaussian effects, the DKI yields more accurate estimates of DT metrics than DTI itself [40], as well as enabling a more comprehensive assessment of the diffusion microenvironment in brain tissue.

Regions of interest (ROIs) were defined for the corpus callosum (CC), fimbria (Fi), external (EC) and internal (IC) capsule, which are WM tracks relevant to age-related changes and in AD pathology. These were manually drawn on the averaged b-value=0 image by a neuropathologist (MFF), using ImageJ (<http://rsb.info.nih.gov/>) [41]. Anatomical guidelines for outlining these regions were determined by comparing anatomical structures in the MRI slices with a standard mouse atlas [42] and verified with the FA maps to ensure correct anatomical location and to avoid contamination of unintended tissue or cerebrospinal fluid (CSF). Slice positioning and sample ROIs for one animal are shown in Figure 1. The mean regional value for each diffusion metric was obtained by averaging over all the voxels within a given ROI using ImageJ.

2.4 Statistical analyses

General linear mixed models (GLMM) were used to model each diffusion metric separately, with age and ROI as predictors. The covariance structure within each mouse was estimated using the direct product of distinct covariance structures for ROI and age. ROI had an unstructured covariance which was inferred from the data, while age had an autoregressive covariance, meaning that the correlation between measurements at the earliest age and those at each successive age became less with time. Since the region-specific metrics represent means of many voxel-specific measures, the observations were weighted based on the inverse of the region-specific standard deviation for each mouse over each diffusion metric; this process generally provided superior model fit based on the Akaike Information Criterion [43] when compared to unweighted or inverse-variance weighted analyses. SAS v9.4 (SAS Institute, Cary, NC) was used to construct the linear mixed models, and R (version3.4.3) was used for graphical purposes. All reported p values were considered statistically significant at $p < 0.05$.

3. Results

Representative parametric maps of all considered diffusion measures for a single anatomical slice from one mouse brain are shown in Figure 2. Note that the diffusivities have units of $\mu\text{m}^2/\text{ms}$, while the other parameters are dimensionless. Both the anisotropies and kurtoses are generally elevated in WM relative to gray matter, reflecting the distinct microstructural characteristics of these two types of brain tissue.

Table 1 shows, for all of the ROIs, the mean values $\pm$ standard deviations of each diffusion measure of each diffusion measure at each imaging time-point. The qualitative trends for the different quantities between time-points one and five are depicted in Figure 3. For the ROIs

studied, we observed that MD, $D_{\parallel}$ and $D_{\perp}$ generally decreased with age, while MK, $K_{\parallel}$ and $K_{\perp}$ generally increased. KFA and FA remained unchanged, except for the Fi region, which showed a trend for increase. Changes in all diffusion measures were more pronounced in the Fi and EC regions.

Figure 4 summarizes results from the GLMMs, showing the estimated percent change for each diffusion metric between time-points one (4.5 months) and five (18 months) for each ROI. In the Fi, significant changes were observed for all but one diffusion parameter (MD, $D_{\perp}$, $D_{\parallel}$, MK, KFA, $K_{\parallel}$ and $K_{\perp}$), while five diffusion parameters (MD, $D_{\perp}$, MK, $K_{\parallel}$ and $K_{\perp}$) reached significance in the EC. In contrast, only one diffusion parameter showed significant changes in the IC (MD) and CC ($K_{\parallel}$). Both the MD and $K_{\parallel}$ had significant changes in three of the four regions, while the FA did not exhibit significant alterations in any of the ROI.

The individual data points together with the average temporal trajectories for all diffusion parameters in each ROI are illustrated in Figure 5. The most pronounced differences are seen in the Fi region, with all measures other than the FA displaying clear changes. Our data suggest that differences relative to the initial time point are already apparent in the Fi when the mice are 9 months-old.

4. Discussion

Significant changes occur for several diffusion measures in WM of 3xTg-AD mice over the age range of 4.5 to 18 months, which demonstrates the ability of dMRI to detect alterations in WM microstructure for this transgenic model associated with aging and/or pathology. As a consequence, dMRI is a viable tool for assessing disease progression and the impact of potential therapies on WM morphology in 3xTg-AD mice, with the advantages of being non-invasive and able to sample the entire brain.

Several biological processes may underlie the longitudinal dMRI changes reported here. Morphological changes such as myelin breakdown, increase in axonal membrane permeability, decrease in the density of myelinated axons and fiber loss are all possible. Increased extracellular space, cell swelling and the presence of inflammatory cells may also have an impact upon water diffusion. However, only a dMRI study with a temporal morphological characterization of this model will be able to identify the specific pathological feature and elucidate the underlying mechanisms for each dMRI metric change and specific brain region.

The observed changes were much more pronounced in some anatomical regions than others. In particular, the Fi and EC were significantly altered in seven and five out of the eight diffusion measures considered, respectively, while only a single diffusion parameter was altered in the IC and in the CC. We also found large variability in the sensitivity of the various diffusion measures with MD and $K_{\parallel}$ being the most sensitive to change, highlighting the advantage of employing multiple parameters.

It should also be noted that all diffusivity measures with significant differences decreased with age, while all kurtosis measures with significant differences increased with age. Since our study did not include a control group it was not possible to determine the extent to which

the observed dMRI changes are directly related to aging rather than to different stages of disease progression or both. However, since age-related changes usually lead to increases in water diffusivity [44–46], and our results showed a decrease in diffusivity metrics associated with an increase in kurtosis metrics, it is likely that a pathological component is associated with these changes. Additionally, since the dMRI changes seem to start when mice are around 9 months old (Figure 5), when both intraneuronal A β and extracellular amyloid deposits are present both in the cortex and hippocampus, it suggests that, at least in part, the WM changes occur along with the progression of AD-like pathology. However, a dMRI study with a temporal morphological characterization of this model is needed to confirm this hypothesis.

Previous dMRI studies have reported similar trends of decrease in diffusivity and increase in kurtosis in the brain using different mouse models of AD [35,36,45,46]. Our diffusion tensor (DT) metrics results are in general concordance with Sun et al. [45], where reduced $D_{\perp}$ with age in the EC of the transgenic group (APPsw) is seen in the results. However, they also reported reduced $D_{\parallel}$ in the CC which contrasts with our finding of no CC change. Also, our results contrast with Sahara et al. [46], where an age-related FA decrease is reported in several WM fiber tracks, including CC and Fi, but our results did not show FA change in any of the regions examined. Additionally, the two DKI studies in a mouse model of AD [35, 36] examined mostly grey matter regions showing an overall increase of MK and K $\parallel$ in the cortex of APP/PS1 mice. The only WM region examined was the CC [36], and they reported an age-related decrease in FA at the genu of the CC and an age-related increase in FA at the body and splenium of the CC. However, it should be noted that important methodological factors distinguish our study from all these prior investigations, making it difficult to directly compare results.

The only other dMRI study assessing the 3xTg-AD mouse model did not find statistically significant changes in WM between transgenic mice and controls [14], but in contrast to our study, no age-related differences in trajectories are reported, only group comparisons. Several other methodological factors distinguish this prior work from ours. In our study, we used only female mice, while in Ref. [14] both male and female mice were used. Since pathology is more variable and far less pronounced in male 3xTg-AD mice [47,48], this may have reduced their ability to detect WM changes. Additionally, ROIs were sampled differently; in Ref. [14], the CC and EC were combined, and the Fi was not assessed. Finally, we used DKI, which provides a more comprehensive characterization of water diffusion than DTI. In fact, more significant differences were observed for the DK measures than for the DT measures, highlighting the additional sensitivity to microstructural changes that DKI provides.

The results presented here reveal, for the first time, age and/or pathology WM related changes in the brain of 3xTg-AD mice using DKI. In our study, the most pronounced changes occurred at the Fi region. Myelinated axons coming from neurons in the subiculum and hippocampus will form the alveus and aggregates to form the Fi, which will proceed upward to form the fornix [49,50]. The fimbria-fornix fibers acts as the major output tract of the hippocampus and play an important role in the formation and consolidation of memories, and they have been shown to be vulnerable to aging as well as AD pathology [30,51–55].

Thus, the decrease in diffusivity metrics associated with an increase in kurtosis metrics in the Fi of the 3xTg-AD mice probably reflects the AD pathology progression in the hippocampus.

The main limitations of this preliminary study are the small number of mice studied and the absence of a normal control group. However, our principal goal was to investigate the sensitivity of dMRI for detecting alterations in WM microstructure at different pathological stages of the 3xTg-AD model, and we found significant diffusion changes over time in this model despite these limitations.

6. Conclusion

We have demonstrated that alterations in WM microstructure due to aging and/or the progression of pathology in 3xTg-AD mice can be detected with dMRI. Thus, dMRI is a viable tool for monitoring the effects of therapeutic interventions on brain tissue in this model. However, changes in diffusion measures were only observed in some anatomical regions, with the most pronounced differences being seen in the fimbria, and the several diffusion measures considered showed a variable sensitivity. The inclusion of kurtosis parameters, made possible by the use of DKI, resulted in more significant differences being observed, and it may therefore be advantageous to employ this extension of DTI in dMRI studies of the 3xTg-AD model.

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Figure 1.

The position of the 15 coronal slices for the dMRI acquisition is shown in the leftmost image. Examples of the four white matter ROIs used in the data analysis are shown on the average b-value=0 image (middle image) and on the FA map (right image). The corpus callosum (CC - blue), fimbria (Fi - orange), external (EC - green) and internal (IC - red) capsule were manually drawn on the b-value=0 image and verified with the FA maps to ensure correct anatomical location.

Figure 2.

Representative dMRI parametric maps of all considered diffusion measures for a single anatomical slice from one mouse brain. The left column shows the DT measures of mean diffusivity (MD), axial diffusivity ($D_{\parallel}$), radial diffusivity ($D_{\perp}$) and fractional anisotropy (FA), while the right column shows the DK measures of mean kurtosis (MK), axial kurtosis ($K_{\parallel}$), radial kurtosis ($K_{\perp}$) and kurtosis fractional anisotropy (KFA). Scale bars: 0–1 for FA and KFA; 0–2 $\mu\text{m}^2/\text{ms}$ for MD, $D_{\parallel}$ and $D_{\perp}$; 0–3 for MK, $K_{\parallel}$, and $K_{\perp}$.

	MD	$D_{\parallel}$	$D_{\perp}$	FA	MK	$K_{\parallel}$	$K_{\perp}$	KFA
Fi	↓	↓	↓	↗	↑	↑	↑	↗
EC	↓	↓	↓	→	↑	↑	↑	→
IC	↘	↘	↘	→	↗	↗	→	→
CC	↘	↘	→	→	↗	↑	↘	→

Figure 3.

Summary of qualitative trends: vertical arrows represent a metric in which all mice exhibited the same trend between time-points one (4.5 months) and five (18 months); diagonal arrows indicate that one mouse had a contrary trend, while horizontal arrows indicate no clear trend. Mean (MD), axial ($D_{\parallel}$) and radial ($D_{\perp}$) diffusivities, fractional anisotropy (FA), mean (MK), axial ($K_{\parallel}$), and radial ($K_{\perp}$) kurtoses and kurtosis fractional anisotropy (KFA). Fimbria (Fi), external capsule (EC), internal capsule (IC) and corpus callosum (CC).

Figure 4.

Estimated percent changes for all diffusion measures over the time interval between 4.5 months and 18 months for each ROI, shown as 95% confidence intervals. All changes were calculated using GLMMs and the full dataset of 5 time points. Significant differences are indicated by * ($p < 0.05$) and ** ($p < 0.01$). Mean (MD), axial ($D_{\parallel}$) and radial ($D_{\perp}$) diffusivities, fractional anisotropy (FA), mean (MK), axial ($K_{\parallel}$), and radial ($K_{\perp}$) kurtoses and kurtosis fractional anisotropy (KFA). Fimbria (Fi), external capsule (EC), internal capsule (IC) and corpus callosum (CC).

Figure 5.

Individual data points and average temporal trajectories for all diffusion measures in each ROI. Pronounced changes are seen in the fimbria (A) and to a somewhat lesser extent in the external capsule (B) for most of the parameters. In contrast, the internal capsule (C) and corpus callosum (D) both showed relatively less variation with time. Mean (MD), axial ($D_{||}$) and radial ($D_{\perp}$) diffusivities, fractional anisotropy (FA), mean (MK), axial ($K_{||}$), and radial ($K_{\perp}$) kurtoses and kurtosis fractional anisotropy (KFA).

Table 1:

Mean value $\pm$ standard deviation for all imaging time-points observed for each diffusion measure by ROI. Mean (MD), axial ($D_{||}$) and radial ($D_{\perp}$) diffusivities, fractional anisotropy (FA), mean (MK), axial ($K_{||}$), and radial ($K_{\perp}$) kurtoses and kurtosis fractional anisotropy (KFA).

Region of Interest	Diffusion Measures (Mean $\pm$ SD)							
	MD ($\mu\text{m}^2/\text{ms}$)	$D_{ }$ ($\mu\text{m}^2/\text{ms}$)	$D_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	FA	MK	$K_{ }$	$K_{\perp}$	KFA
Fimbria (Fi)								
	0.96 $\pm$ 0.03	1.68 $\pm$ 0.07	0.60 $\pm$ 0.03	0.58 $\pm$ 0.02	1.16 $\pm$ 0.03	0.85 $\pm$ 0.0	1.86 $\pm$ 0.0	0.58 $\pm$ 0.0
4.5 months						0	5	2
	0.94 $\pm$ 0.02	1.68 $\pm$ 0.03	0.57 $\pm$ 0.02	0.60 $\pm$ 0.01	1.18 $\pm$ 0.04	0.84 $\pm$ 0.0	1.96 $\pm$ 0.0	0.60 $\pm$ 0.0
9 months						3	8	3
	0.91 $\pm$ 0.02	1.64 $\pm$ 0.06	0.55 $\pm$ 0.01	0.60 $\pm$ 0.02	1.22 $\pm$ 0.05	0.89 $\pm$ 0.0	2.04 $\pm$ 0.1	0.62 $\pm$ 0.0
12 months						2	2	1
	0.89 $\pm$ 0.05	1.60 $\pm$ 0.07	0.53 $\pm$ 0.04	0.61 $\pm$ 0.01	1.24 $\pm$ 0.05	0.91 $\pm$ 0.0	2.11 $\pm$ 0.0	0.63 $\pm$ 0.0
15 months						4	6	4
	0.88 $\pm$ 0.02	1.57 $\pm$ 0.03	0.53 $\pm$ 0.02	0.60 $\pm$ 0.01	1.26 $\pm$ 0.04	0.94 $\pm$ 0.0	2.05 $\pm$ 0.0	0.62 $\pm$ 0.0
18 months						3	7	2
External Capsule (EC)								
	0.79 $\pm$ 0.03	1.03 $\pm$ 0.03	0.68 $\pm$ 0.02	0.30 $\pm$ 0.01	0.89 $\pm$ 0.04	0.81 $\pm$ 0.0	0.98 $\pm$ 0.0	0.42 $\pm$ 0.0
4.5 months						3	6	3
	0.78 $\pm$ 0.04	1.00 $\pm$ 0.06	0.66 $\pm$ 0.03	0.30 $\pm$ 0.01	0.90 $\pm$ 0.03	0.80 $\pm$ 0.0	1.01 $\pm$ 0.0	0.42 $\pm$ 0.0
9 months						2	5	3
	0.78 $\pm$ 0.04	1.01 $\pm$ 0.05	0.66 $\pm$ 0.04	0.30 $\pm$ 0.01	0.92 $\pm$ 0.05	0.81 $\pm$ 0.0	1.05 $\pm$ 0.0	0.42 $\pm$ 0.0
12 months						1	7	5
	0.76 $\pm$ 0.03	0.99 $\pm$ 0.04	0.64 $\pm$ 0.02	0.31 $\pm$ 0.01	0.93 $\pm$ 0.02	0.83 $\pm$ 0.0	1.03 $\pm$ 0.0	0.44 $\pm$ 0.0
15 months						2	4	3
	0.75 $\pm$ 0.01	0.98 $\pm$ 0.02	0.64 $\pm$ 0.01	0.30 $\pm$ 0.01	0.96 $\pm$ 0.03	0.88 $\pm$ 0.0	1.07 $\pm$ 0.0	0.42 $\pm$ 0.0
18 months						4	4	1
Internal Capsule (IC)								
	0.77 $\pm$ 0.02	1.26 $\pm$ 0.03	0.53 $\pm$ 0.02	0.50 $\pm$ 0.02	1.22 $\pm$ 0.01	0.96 $\pm$ 0.0	1.69 $\pm$ 0.0	0.53 $\pm$ 0.0
4.5 months						3	4	3
	0.77 $\pm$ 0.01	1.27 $\pm$ 0.02	0.52 $\pm$ 0.01	0.51 $\pm$ 0.01	1.23 $\pm$ 0.03	0.93 $\pm$ 0.0	1.73 $\pm$ 0.0	0.53 $\pm$ 0.0
9 months						2	6	3
	0.77 $\pm$ 0.01	1.26 $\pm$ 0.02	0.52 $\pm$ 0.01	0.51 $\pm$ 0.01	1.21 $\pm$ 0.01	0.93 $\pm$ 0.0	1.74 $\pm$ 0.0	0.54 $\pm$ 0.0
12 months						2	1	2
	0.74 $\pm$ 0.01	1.22 $\pm$ 0.02	0.51 $\pm$ 0.01	0.51 $\pm$ 0.02	1.21 $\pm$ 0.06	0.94 $\pm$ 0.0	1.72 $\pm$ 0.1	0.55 $\pm$ 0.0
15 months						5	1	2
	0.75 $\pm$ 0.01	1.22 $\pm$ 0.05	0.52 $\pm$ 0.02	0.50 $\pm$ 0.03	1.26 $\pm$ 0.09	1.00 $\pm$ 0.0	1.74 $\pm$ 0.1	0.53 $\pm$ 0.0
18 months						6	1	5
Corpus Callosum (CC)								
	0.77 $\pm$ 0.03	1.01 $\pm$ 0.03	0.65 $\pm$ 0.03	0.40 $\pm$ 0.02	1.00 $\pm$ 0.05	0.94 $\pm$ 0.0	1.23 $\pm$ 0.1	0.54 $\pm$ 0.0

Region of Interest	Diffusion Measures (Mean $\pm$ SD)							
	MD ($\mu\text{m}^2/\text{ms}$)	D ($\mu\text{m}^2/\text{ms}$)	D _⊥ ($\mu\text{m}^2/\text{ms}$)	FA	MK	K	K _⊥	KFA
4.5 months						6	1	4
	0.78 $\pm$ 0.02	1.05 $\pm$ 0.03	0.64 $\pm$ 0.02	0.42 $\pm$ 0.02	1.04 $\pm$ 0.03	1.01 $\pm$ 0.0	1.26 $\pm$ 0.0	0.54 $\pm$ 0.0
9 months						3	7	4
	0.78 $\pm$ 0.03	1.03 $\pm$ 0.04	0.65 $\pm$ 0.02	0.41 $\pm$ 0.02	1.05 $\pm$ 0.03	1.00 $\pm$ 0.0	1.23 $\pm$ 0.0	0.52 $\pm$ 0.0
12 months						5	9	1
	0.74 $\pm$ 0.01	0.99 $\pm$ 0.01	0.62 $\pm$ 0.01	0.42 $\pm$ 0.01	1.02 $\pm$ 0.04	1.01 $\pm$ 0.0	1.19 $\pm$ 0.0	0.55 $\pm$ 0.0
15 months						3	2	3
	0.75 $\pm$ 0.02	0.99 $\pm$ 0.01	0.64 $\pm$ 0.03	0.42 $\pm$ 0.02	1.04 $\pm$ 0.06	1.05 $\pm$ 0.0	1.15 $\pm$ 0.0	0.53 $\pm$ 0.0
18 months						6	8	5