Age-related differences in white matter: Comparing fixel based and tensor based analyses

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Conflict of Interest

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Abstract

Older adults tend to perform worse on cognitive, behavioral, motor, and sensory tasks compared to younger adults, and differences in white matter that may be associated with this phenomenon are being actively investigated. Most prior studies of white matter differences between older and younger adults have analyzed diffusion weighted images using diffusion tensor imaging (DTI) analysis. But DTI results can be affected by many different factors (e.g., fiber density, fiber cross-section, crossing fibers) that are difficult to distinguish, making the interpretation of these results challenging. Recently, new fixel-based analysis (FBA) techniques have been developed that address some of these concerns, but these techniques have not yet been applied in the domain of aging. In this study, we used both DTI and FBA to analyze differences in white matter in a large sample of older and younger healthy adults. Both analysis methods identified age differences in forceps minor, fornix, bilateral internal capsule, and bilateral inferior fronto-occipital fasciculi, but the FBA results provided novel insights into the underlying structural differences. Furthermore, DTI analysis identified differences in superior longitudinal fasciculus that are not reflected in fiber density or cross-section and may instead be due to differences in crossing fiber geometry. Finally, the FBA results identified clearer differences in limbic white matter than did the DTI analysis. It also provided stronger evidence of an anterior-posterior asymmetry and segment-specific variations in white matter differences between older and younger adults. These results demonstrate the power of fixel-based analysis and provide novel insights into some of the major white matter differences associated with healthy aging.

Keywords: Aging, white matter, diffusion, fixel, tensor, tractography

Introduction

The brain undergoes a series of structural and functional changes from young to old adulthood. In this study, we used Diffusion Magnetic Resonance Imaging (dMRI) to analyze white matter *in vivo* and to study differences in specific tracts between older and younger adults. By measuring the diffusion of water molecules along multiple axes throughout the brain, dMRI can be used to infer the geometry and structural properties of white matter fiber bundles. Because white matter fascicles preferentially restrict diffusion perpendicular to their orientation, the direction and strength of diffusion in individual voxels can be used to estimate the orientations and microstructural properties (e.g., fiber density) of white matter pathways that traverse them.

One of the most widely used models for characterizing diffusion within individual voxels is the diffusion tensor (DT) model. This approach models diffusion as a three-dimensional zero-mean Guassian distribution, whose parameters can be used to estimate the average magnitude (mean diffusivity; MD), primary orientation (principal diffusion direction; PDD), and anisotropy (fractional anisotropy; FA) of local diffusion. Of particular interest in white-matter neuroimaging is the FA metric, which estimates the directional cohesion of diffusion within a voxel, and is therefore sensitive to changes in fiber density, cohesion, and myelination when only a single fiber pathway traverses a voxel. Unfortunately, FA's sensitivity to these many different factors makes it challenging to identify which factor (or factors) is responsible for any observed effects. A previous study found that a general factor of FA was able to explain ~45% of individual differences for all tracts examined, which may suggest that FA is limited in its ability to reflect and describe complex microstructural phenomena (Penke et al., 2010). Furthermore, because the DT model does not distinguish between distinct fiber pathways that can traverse a voxel in different directions, the FA metric can fail to provide meaningful information about these microstructural properties when crossing fibers are present, conflating microstructural differences with differences in local multi-fiber geometry. And crossing fibers appear to be the rule rather than the exception: it is estimated that approximately 60-90% of white matter voxels contain multiple directionally distinct fiber populations (Jeurissen, Leemans, Tournier, Jones, & Sijbers, 2013), leaving open the possibility that previously observed age group differences in DT metrics may partially reflect differences in local multi-fiber geometry, rather than microstructural differences more directly related to transmission capacity along particular pathways.

Table 1 provides a summary of seminal studies which used DTI to investigate white matter differences between age groups. They were also chosen for being similar to our study in that they compare cohorts of younger and older adults rather than studying differences with a continuous age scale. As shown in the main findings column, there is variability in the tracts that have been found to exhibit lower FA in older adults. The genu of the corpus callosum was found to have lower FA in older adults in most studies, while cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, internal and external capsule, fornix, corona radiata, forceps minor, and sagittal striatum were found to show lower FA in older adults in at least two studies. The summary of these results exemplify the limitations of reproducibility with DTI.

Furthermore, using DTI alone it is not possible to determine the types of structural differences (e.g., in fiber density versus cross-section) that underlie the observed differences in FA. To understand these underlying factors, it would be preferable to have a model that can separate the different structural features reflected in FA.

References	Subjects	Age range (mean ± SD)	Image Acquisition	Image Analysis	Main Findings (old relative to young)
Bennett, Madden, Vaidya, Howard, and Howard (2010)	14 younger adults 14 older adults	18-20 (18.9 ± 0.7) 63-72 (67.6 ± 3.1)	3 T 35 diffusion directions 2.5 mm slice	TBSS	Lower FA: Frontal, posterior pericallosal, superior longitudinal fasciculus, sagittal striatum, genu of the corpus callosum, external capsule, fornix, anterior pericallosal, anterior/superior corona radiata, cerebellum, cerebral peduncle Greater FA: None
Burzynska et al. (2010)	80 younger adults 63 older adults	20–32 (25.7 ± 3.2) 60–71 (64.8 ± 2.9)	1.5 T 12 diffusion directions 2.5 mm slice	TBSS	Lower FA: anterior, superior and posterior corona radiata, white matter of the superior, inferior, middle, frontal and straight gyri, white matter of the precuneus and superior parietal lobule, cingulum (mainly dorsal), fornix, forceps minor and major, external capsule, internal capsule, sagittal striatum, parahippocampal white matter Greater FA: None
Davis et al. (2009)	20 younger adults 20 older adults	n/a (20.04 ± 2.5) n/a (68.89 ± 5.3)	3 T 15 diffusion directions 2.0 mm slice	TG TBSS	Lower FA: genu and splenium of the corpus callosum, the cingulum bundle, inferior longitudinal fasciculus, uncinate fasciculus Greater FA: None
Giorgio et al. (2010)	37 younger adults 19 mid-adults 10 older adults	23.0–40.2 (n/a) 41.0-59.6 (n/a) 60.0-81.6 (n/a)	1.5 T 60 diffusion directions 2.5 mm slice	TBSS VBM	Lower FA: Anterior thalamic radiations, external capsule, anterior limb of internal capsule, cerebral peduncle and cerebellum corona radiata, superior

					longitudinal fasciculus, forceps minor, inferior fronto-occipital fasciculus Greater FA: None
Hugenschmidt et al. (2008)	20 younger adults 21 mid-adults 23 older adults	18–38 (28.30 ± 6.3) 39–64 (47.57 ± 7.6) 65–90 (71.17 ± 4.3)	1.5 T 15 diffusion directions 3.0 mm slice	ROI VBM	Lower FA: Genu and body of the corpus callosum, forceps minor, superior and inferior frontal gyrus, centrum semiovale, optic radiations and external capsule, corticospinal tract Greater FA: n/a
Pfefferbaum, Adalsteinsson, and Sullivan (2005)	10 younger adults 10 older adults	22–37 (28.6 ± n/a) 65–79 (72.2 ± n/a)	3 T 6 diffusion directions 2.5 mm slice	VBM	Lower FA: Superior longitudinal fasciculus, inferior longitudinal fasciculus, anterior cingulate bundle, middle frontal gyrus, frontal forceps and genu Greater FA: n/a
Salat et al. (2005)	15 younger adults 9 mid-adults 14 older adults	21–37 (26.3 ± n/a) 42–59 (51.8 ± n/a) 65–76 (70.9 ± n/a)	1.5 T 6 diffusion directions 2 mm slice	ROI	Lower FA: Genu of the corpus callosum, bilateral deep frontal white matter, posterior limb of internal capsule, medial orbitofrontal white matter and posterior periventricular Greater FA: n/a

Table 1 Summary of previous DTI studies of differences between age groups adapted from Yap et al. (2013). Regions of interest (ROI), Tract-based spatial statistic (TBSS), Tractography (TG), Voxel-based morphometry (VBM)

Fixel-based analysis (FBA) is a recently developed model that purports to do exactly that (Raffelt et al., 2012). Unlike the DT model, FBA relies on a spherical harmonic representation of diffusion that can more readily represent complex multi-fiber geometry, allowing for anatomically-informative metrics to be separately estimated for distinct fiber populations within a voxel. Specifically, fixel-based analysis utilizes constrained spherical deconvolution (CSD) to estimate fiber orientation distribution functions (fODFs) within each voxel, and then segments fODFs into directionally distinct lobes associated with distinct fiber populations (“fixels”) within a voxel. This allows for the diffusion anisotropy associated with individual fiber segments to be

estimated independently by measuring the size of their respective fODF lobes, effectively deconfounding local multi-fiber geometry and tissue microstructure metrics. Additionally, FBA can be used to analyze the morphometrically-estimated cross-sectional size of fiber pathways, allowing for independent analysis of microstructural measures of fiber density (FD) and macrostructural measures of fiber cross-section (FC) (Raffelt et al., 2017). It can also be used to estimate the overall size of a pathway, by analyzing the product of fiber density and cross-section (FDC).

In this study we analyzed differences in white matter between older and younger adults, using both the FA parameter from traditional DTI and the more specific FD, FC, and FDC measures from the FBA model. Doing so shed light on the specific structural features that underlie differences in white matter that have been identified using DTI. It also revealed differences that may be primarily due to variations in crossing fibers as well as novel differences that have not previously been reported.

Methods

Participants

Participants for this study were recruited as part of the Michigan Neural Distinctiveness (MiND) study (Gagnon et al., 2019). All participants were healthy (no debilitating physical conditions, mental illness, or head trauma), were right-handed and were free of significant cognitive impairment (Montreal Cognitive Assessment score above 22) (Nasreddine et al., 2005). Participants were separated into 25 younger adults (19-29 years old) and 45 older adults (65-87 years old). Participants were excluded if they had motor control or hearing problems, had current depression or anxiety, history of drug or alcohol abuse or drank more than 4-6 alcoholic beverages per week (4 for women and 6 for men). Complete information regarding exclusion criteria is reported in Gagnon et al. (2019). All study procedures were approved by the University of Michigan Medical School Institutional Review Board. Participant demographics are displayed in Table 2.

	Younger Adults n = 25	Older Adults n = 45	Statistic
Mean age (SD)	23.32 (3.06)	70.69 (4.95)	t = 43.35 p = < .001
Gender (%)			
Male	10 (40)	17 (37.78)	χ^2= 0.034
Female	15 (60)	28 (62.22)	p = 0.855
Mean years of education (SD)	15.72 (1.34)	16.91 (2.10)	t = 2.56 p = 0.013

Table 2. Age, gender, and education of the study participants.

Imaging Acquisition and Processing

MRI data were collected using a 3T General Electric Discovery Magnetic Resonance System with an 8-channel head coil at the University of Michigan's Functional MRI Laboratory. The dMRI data were collected with a diffusion-weighted 2D dual spin echo pulse sequence with the following parameters: Repetition Time (TR) = 7250 ms; Echo Time (TE) = 2.5 ms; Field of View (FOV) = 240 × 240 mm; 32 diffusion directions; 60 axial slices with thickness = 2.5 mm (0.9375 mm in-plane resolution) and 0.1 mm spacing. Five volumes without diffusion weighting (b = 0 s/mm^2) and 32 diffusion-weighted volumes (b = 1000 s/mm^2) were collected. Acquisition time was approximately 10 minutes. Two scans were collected with the previously described properties with opposite phase-encoding. T1-weighted structural images were collected with the following parameters: TR = 3173.1 ms; TE = 24.0 ms; Inversion Time (TI) = 896 ms; flip angle = 111°; FOV = 220 × 220 mm; 43 axial slices with thickness = 3 mm and no spacing; acquisition time = 100 seconds.

Diffusion Magnetic Resonance Images (dMRIs) were preprocessed using MRtrix (https://mrtrix.readthedocs.io/en/latest/fixel_based_analysis/st_fibre_density_cross-section.html). Susceptibility distortion, motion, and eddy current correction were performed using FSL's eddy/topup tools (Jenkinson et al., 2012). Bias field correction was performed using ANTS N4 (http://picsl.upenn.edu/software/ants/). We did not include a denoising or unringing step. dMRIs were intensity normalized across subjects based on the median b = 0 s/mm^2 intensity within a white matter mask and resampled to an isotropic voxel size of 1.3 mm using b-spline interpolation. Constrained spherical deconvolution (CSD; "Tournier" algorithm) was used to compute fiber orientation distributions (FODs) (Tournier, Calamante, & Connelly, 2007). A group average response was used to estimate FODs in all subjects, as described in Raffelt et al. 2012 and registration was performed. A white matter template fixel mask was generated with a peak amplitude threshold of 0.15. Whole brain probabilistic tractography was then performed on the FOD template generating 20 million streamlines and spherical-deconvolution informed filtering of tractograms (SIFT) was applied with an output of 2 million streamlines (Smith, Tournier, Calamante, & Connelly, 2013). SIFT removes individual streamlines so that the density of reconstructed connections is proportional to the fiber density as estimated by the diffusion model, providing a biologically relevant estimation of the density of white matter axons connecting two regions.

A five-tissue-type (5TT) image was generated from the T1-weighted structural image using FSL. Volumes from the 5TT image were used to estimate regions of CSF and were included as masks in further analyses to minimize partial voluming effects.

DMRI Analysis. We performed whole brain analyses of FA and of the MRtrix-derived measures fiber density (FD), fiber cross section (FC), and fiber density and cross section (FDC). We also

performed tract-specific analyses.

Whole-brain DTI and Fixel-Based Analysis

Diffusion tensor images were generated from group-registered diffusion weighted images using an iteratively reweighted linear least squares estimator. Fractional anisotropy (FA) maps were then created for each subject. Whole-brain voxel-based analysis was then performed to identify voxels in which FA was significantly different in the young vs. the older participants (with threshold-free cluster enhancement, default parameters: dh=0.1, e=0.5, h=2; 5000 permutations for familywise error rate correction) (Smith & Nichols, 2009; Holmes, Blair, Watson, & Ford, 1996).

We also performed a similar analysis using fixel-based methods. Fiber density (FD), fiber cross section (FC), and fiber density and cross section (FDC) were calculated in every fixel for each subject and older adults were statistically compared to younger adults at each white matter fixel. Connectivity-based fixel enhancement (CFE) using 2 million streamlines and default parameters (smoothing = 10 mm FWHM, C = 0.5, E = 2, H = 3; taken from Raffelt et al. 2015) was used to analyze FBA metrics. The non-parametric permutation included 5000 permutations. When diffusion sensitivity is high (b > 2000 s/mm2), the integral over an fODF lobe is proportional to fiber density within an associated fiber population. With less diffusion sensitization, as used in this study (b = 1000 s/mm2), this metric is also sensitive to extra-axonal diffusion, and therefore may also reflect axonal hindrance of extra-cellular diffusion and other microstructural factors captured by FA in single-fiber voxels**.**

Tract-Specific Analyses

We also performed tract-specific analyses using both DTI-based and FBA-based metrics. We selected sixteen major tracts for analysis, including “canonical” pathways included in most white matter parcellations and previous studies of age group differences in the DTI literature. Wakana et al. (2007) and Catani and Schotten (2012) were used as anatomical guidelines to manually select regions of inclusion and exclusion. The white matter template from FBA was used for ROI placement so that all subjects’ data were registered beforehand. Tracts of interest were generated from the whole brain tractogram generated for FBA. Tracts were thresholded to only include fixels that had at least 1% of total streamlines associated with them. The following tracts were generated: cingulum (separated into retrosplenial, subgenual cingulum and parahippocampal cingulum as described in Jones, Christiansen, Chapman, and Aggleton (2013) and the parahippocampal cingulum was further separated into parietal and temporal components), corticospinal tract (separated into superior and inferior), forceps major, forceps minor, fornix, inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), internal capsule, superior longitudinal fasciculus (SLF, separated into SLF I, II and III as described in Schotten et al. (2011)) and uncinate fasciculus. SLF I projects to the parietal precuneus and supplementary motor area, SLF II projects to the posterior region of the inferior parietal lobule and the lateral aspect of the superior and middle frontal gyrus and SLF III projects

to the inferior parietal lobule and the posterior region of the inferior frontal gyrus (Catani & Schotten, 2012). The parahippocampal cingulum and corticospinal tract were each divided into two components to facilitate comparison with our whole-brain analyses, which indicated distinct local effects along these pathways. Mean FA, FD, FC and FDC were calculated for each tract bilaterally. Metrics were compared between the two age groups with a Matlab script using 10,000 permutations to correct for multiple comparisons across tracts.

Results

Whole Brain DTI Analysis

Figure 1 displays streamlines corresponding to white matter voxels that exhibited a significantly lower FA value in older adults compared to younger adults. The streamlines are colored by the percent reduction in older compared to younger adults. The lower FA for older adults is widespread and includes tracts such as forceps minor, fornix, bilateral IFOF, bilateral anterior internal and external capsule, and bilateral SLF I and III. The percent reduction in FA of these tracts between older and younger adults is generally around 30%, reaching as much as 50% in regions like the fornix and genu of the corpus callosum.

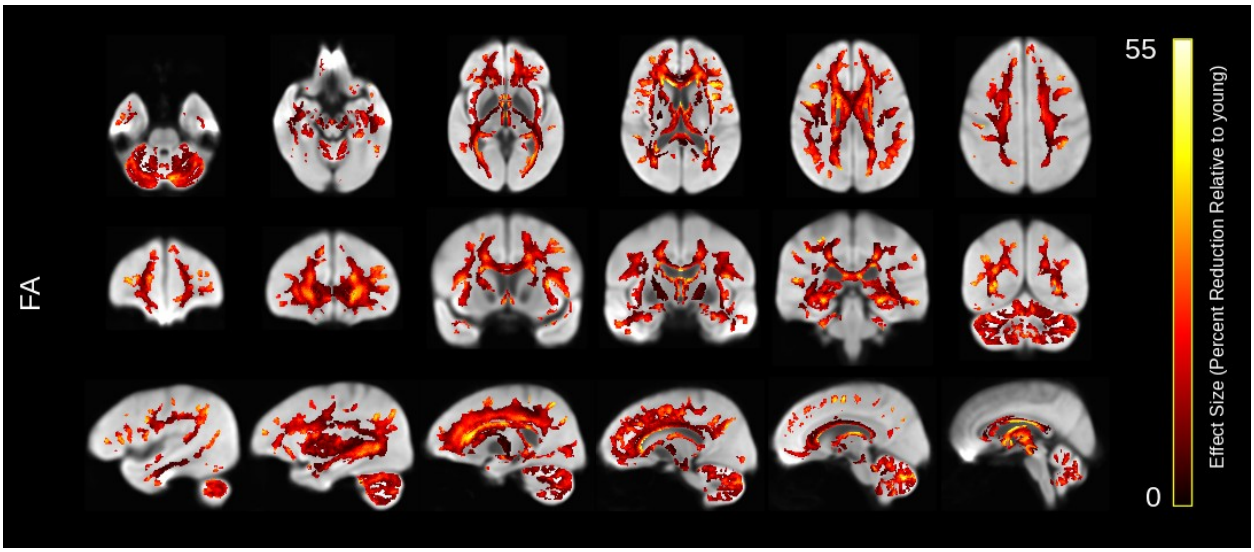

Figure 1. Lower white matter fractional anisotropy (FA) in older compared to younger adults. Colors represent streamlines corresponding to voxels in which FA was significantly lower in the older compared with the younger group, with brighter colors representing greater differences.

Figure 2 displays streamlines corresponding to white matter voxels that exhibited significantly greater FA in older adults compared to younger adults. The streamlines are colored by the percent increase in the old relative to the young. The greater FA for older adults is more limited and occurs in the superior cerebellar peduncles, external capsule and cingulum. The percent increase in FA of these tracts for older compared to younger adults is generally around 30%, reaching as high as 115% in regions like external and internal capsule.

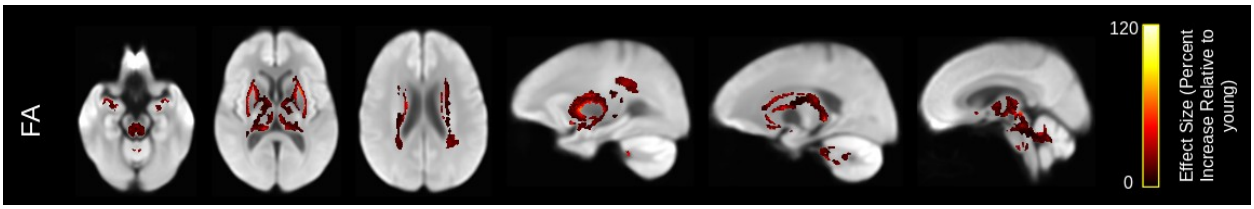

Figure 2. Greater white matter fractional anisotropy (FA) in older compared to younger adults. Colors represent streamlines corresponding to voxels in which FA was significantly greater in the older compared with the younger group, with brighter colors representing greater differences.

Tract-Specific DTI Analysis

In addition to the whole brain analyses just described, we also performed tract-specific analyses on 16 tracts, which were chosen to reflect "canonical" pathways included in most white matter parcellations and previous studies in the DTI literature comparing age groups. The parahippocampal cingulum and cerebrospinal tract were further divided based on our whole-brain analysis, which suggested the presence of distinct differences along these pathways. Figure 3 displays the specific tracts that we analyzed.

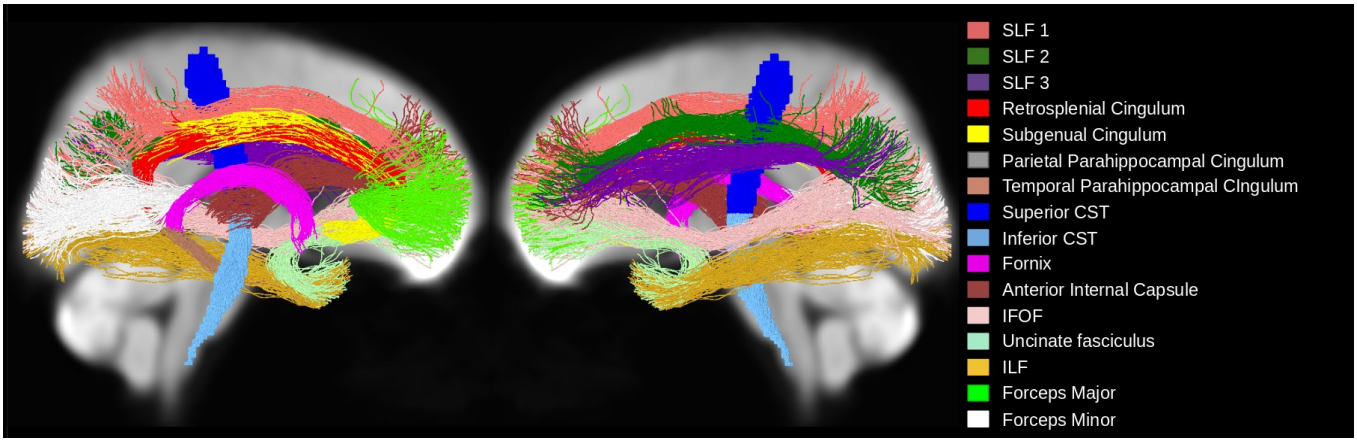

Figure 3. The 16 tracts that were included in the tract-specific analyses.

Figure 4 displays tracts where the tract-specific analysis identified significantly lower mean FA for older compared to younger adults based on permutation testing. All the streamlines of a specific tract are colored by the effect size (Cohen's d) throughout the tract. Similar to the whole-brain analysis, FA was still significantly lower in older vs. younger adults in forceps minor, fornix, bilateral IFOF, bilateral internal capsule and bilateral SLF I, II and III. FA was also significantly lower in bilateral ILF, bilateral parietal parahippocampal cingulum and right uncinate fasciculus. Mean FA was greater in older adults compared to younger adults in right subgenual and retrosplenial cingulum (not shown).

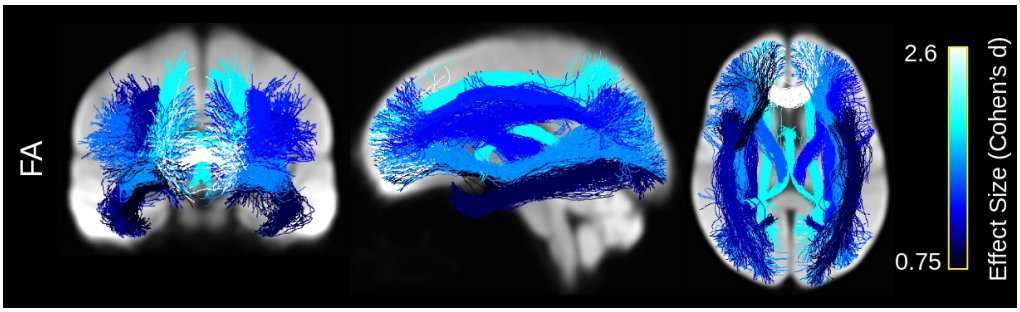

Figure 4. Glass brain views showing white matter tracts in which fractional anisotropy (FA) was significantly lower in the older vs. younger participants. Brighter colors represent greater effect size.

Whole Brain Fixel-Based Analysis

Figure 5 displays streamlines corresponding to white matter fixels that exhibited significantly lower FD (top row), FC (middle row) and FDC (bottom row) in older adults compared to younger adults. The streamlines are colored by the percent reduction in older compared to younger adults. Fiber density was significantly lower in the fornix, bilateral anterior internal capsule, forceps minor, body of the corpus callosum and corticospinal tract of older adults. Fiber cross-section was most significantly lower in the cingulum and forceps minor. FDC was significantly lower in the anterior and subcortical structures such as forceps minor, anterior internal capsule, subgenual cingulum, fornix and IFOF. The percent reduction in the metrics of these tracts for older compared to younger adults is generally around 30%, reaching as much as 55% in regions like the fornix for FD. In general, the FBA-based metrics exhibited a gradient along the anterior-posterior axis.

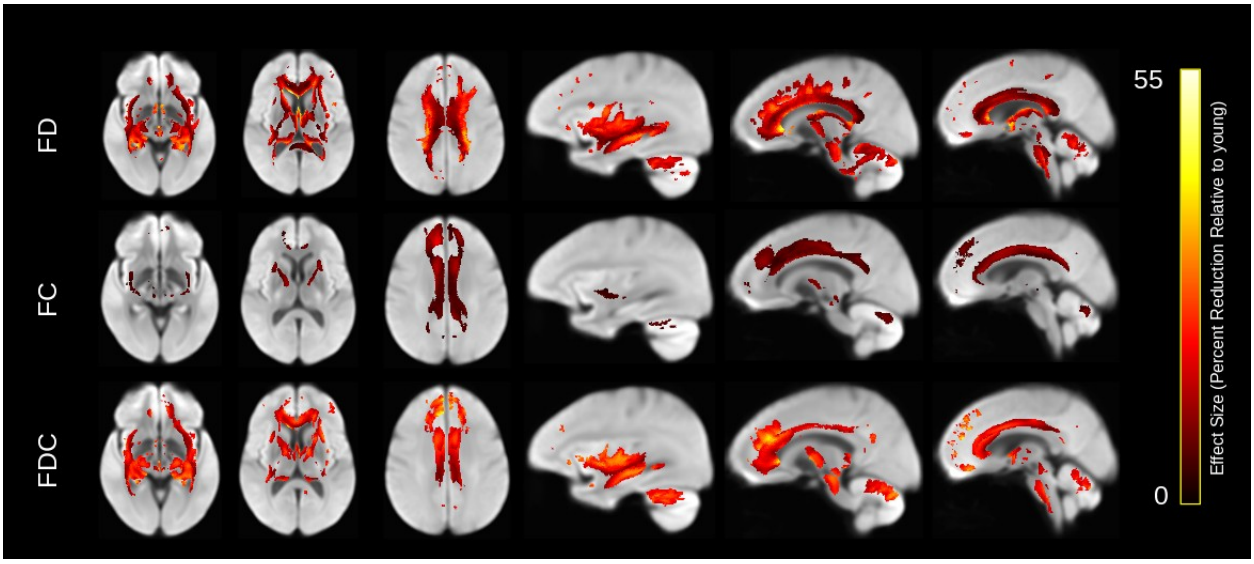

Figure 5. Lower white matter fiber density (FD, top row), fiber cross-section (FC, middle row), and the product of fiber density and cross-section (FDC, bottom row) in older compared to younger adults. Colors represent streamlines corresponding to fixels in which each measure was significantly lower in the older compared with the younger group, with brighter colors representing greater differences.

Figure 6 displays streamlines corresponding to white matter fixels that exhibited significantly

greater FD (top row), FC (middle row) and FDC (bottom row) in older adults compared to younger adults. The streamlines are colored by the percent increase in the old relative to the young. FD was greater in SLF I, and FC was greater in forceps major and the body of the corpus callosum. FDC was only greater in forceps major.

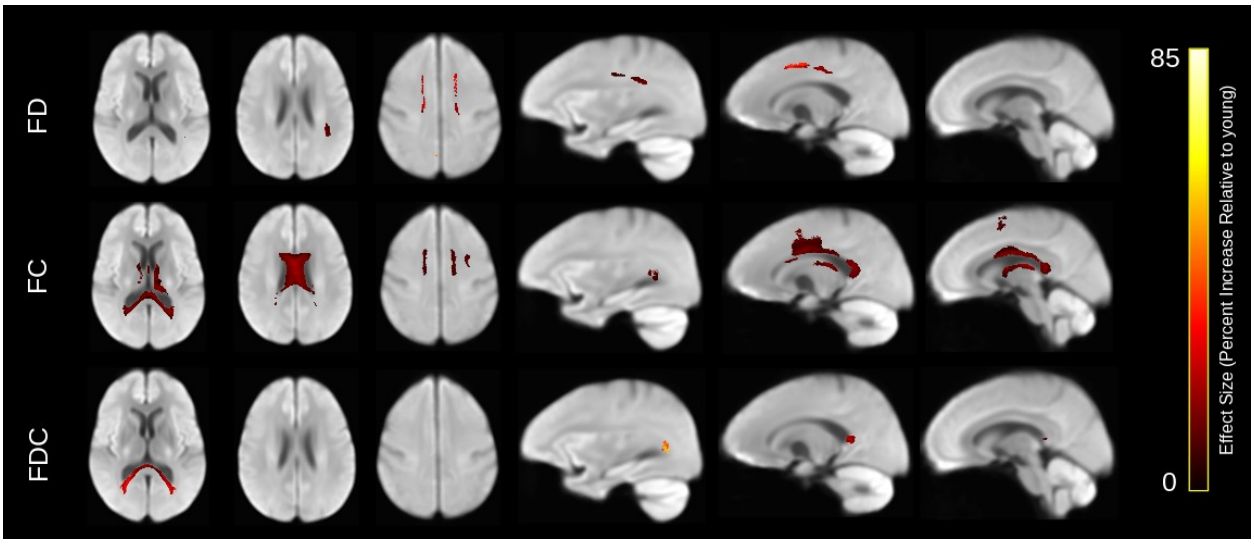

Figure 6. Greater white matter fiber density (FD, top row), fiber cross-section (FC, middle row), and the product of fiber density and cross-section (FDC, bottom row) in older compared to younger adults. Colors represent streamlines corresponding to fixels in which each measure was significantly greater in the older compared with the younger group, with brighter colors representing greater differences.

Tract-Specific Fixel-Based Analysis

Figure 7 displays tracts where the tract-specific analysis identified a significantly lower mean FD, FC or FDC for older adults compared to younger adults based on permutation testing. All the streamlines of a specific tract are colored by the effect size (Cohen's d) throughout the tract. FD was significantly lower in older adults in bilateral temporal and right parietal parahippocampal cingulum, bilateral inferior corticospinal, forceps minor, fornix, bilateral IFOF, bilateral internal capsule and left uncinate fasciculus. FC was significantly lower in older adults in bilateral retrosplenial and subgenual cingulum, bilateral inferior corticospinal and bilateral SLF I. FDC was significantly lower in older adults in bilateral retrosplenial, subgenual and temporal parahippocampal cingulum, bilateral inferior corticospinal, forceps minor, fornix, right IFOF, right ILF, bilateral internal capsule and bilateral uncinate fasciculus. FDC was not significantly lower in the posterior regions of right ILF and right IFOF in FBA, and so it is possible the low effect size for these regions is attributable to tract-specific analysis occurring across an entire tract when the effect may be highest in a particular segment. FD was greater in older adults in left SLF I and FC was greater in older adults in forceps major, and fornix (not shown).

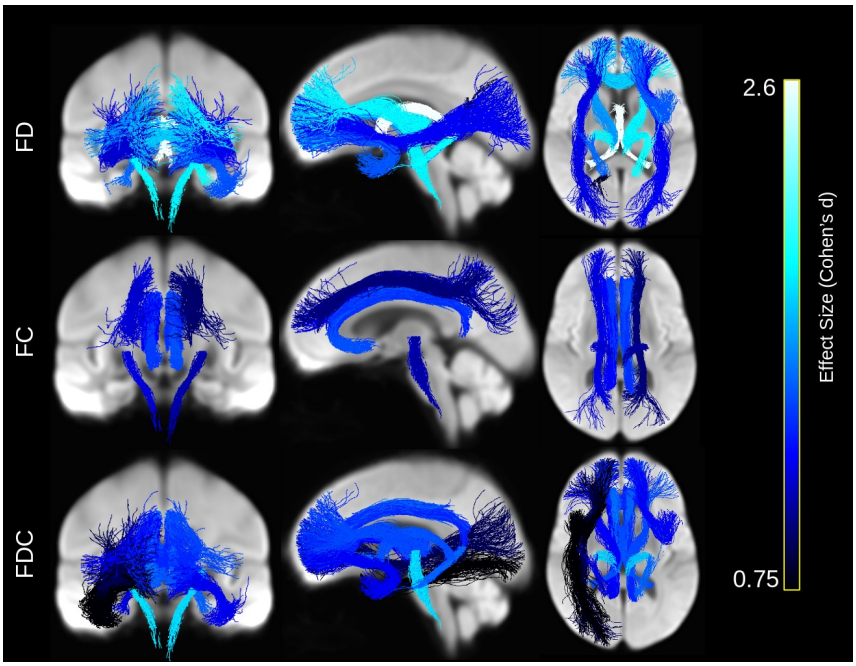

Figure 7. Glass brain views showing white matter tracts in which fiber density (FD, top row), fiber cross-section (FC, middle row), and the product of fiber density and cross-section (FDC, bottom row) was significantly lower in the older vs. younger participants. Brighter colors represent greater effect size.

Discussion

In this paper, we analyzed differences in white matter between older and younger adults using two different approaches: a traditional DTI analysis based on fractional anisotropy (FA) and a fixel-based approach that estimated fiber density (FD), fiber cross-section (FC), and the product of fiber density and cross-section (FDC). The DTI analysis identified lower FA in older adults in forceps minor, fornix, bilateral IFOF, bilateral anterior internal and external capsule, and bilateral SLF I and III. A follow-up tract-specific analysis found significantly lower FA in older adults in many of the same tracts: forceps minor, fornix, bilateral IFOF, bilateral internal capsule and bilateral SLF I, II and III. These age differences in FA could reflect a variety of structural differences and disentangling these is not possible using traditional DTI analysis. In contrast, fixel-based analysis provides estimates of both fiber density and cross-section and can therefore provide novel insights. For example, FA and FD were both lower in forceps minor and anterior internal capsule, suggesting that these differences are due to lower fiber density in older adults rather than from differences in other factors that might affect FA. For forceps minor, both fiber density and cross-section were lower in older adults.

FA was also lower for older adults in the SLF (I and II in the whole-brain analysis and I, II and III in the tract-specific analyses), but interestingly this difference was not observed in the fixel-based analysis of fiber density or fiber cross-section (except that cross-section was lower in SLF I in the tract-specific, but not whole-brain, analysis). Most of the observed differences in SLF are therefore likely due to some other factor. One plausible candidate might be differences

between older and younger adults in the angle, relative size, or proportion of crossing fibers in the voxels traversed by the SLF. Rokem et al. (2015) showed that centrum semiovale, which includes the intersection of the SLF, CST, and corpus callosum, is one of the regions where the diffusion tensor model is least accurate due to the presence of crossing fibers. It is therefore possible that the observed lower FA in SLF is due to a difference in the geometry or proportion of crossing fibers in the centrum semiovale rather than structural differences in the SLF itself.

We observed unexpected greater FA in the superior cerebellar peduncles, external capsule and cingulum of older adults. Kanaan, Allin, Picchioni, Shergill, and Mcguire (2016) previously found greater FA in the superior cerebellar peduncles of older adults compared to younger adults. These findings may suggest that from young to old adulthood regions that experience ongoing development and continual use (such as cerebellar peduncles) may become strengthened into older adulthood.

The fixel-based analysis also uncovered differences that were not observed in the DTI analyses. In particular, fiber cross-section and the product of fiber density and cross-section were both significantly lower in the older group in retrosplenial and subgenual cingulum (in both the whole-brain and tract-specific analysis), but FA (and fiber density) were not. FA may not be sensitive to these kinds of structural differences, or perhaps a difference in some other structural feature masked this effect.

We also observed greater fiber cross-section in the body of the corpus callosum and part of forceps major in older compared to younger adults, but lower fiber density in these same regions. This may explain why this region does not exhibit a significantly lower FDC in older adults compared to younger adults, as the greater fiber cross-section may mask the lower fiber density. Similarly, in the fornix body, cross-section was greater in older adults while density and FDC was lower. These results are consistent with previous findings that older brains show a loss of small diameter axonal fibers, but not larger diameter fibers (Aboitiz, Scheibel, Fisher, & Zaidel, 1992; Bowley, Cabral, Rosene, & Peters, 2010; Marner, Nyengaard, Tang, & Pakkenberg, 2003).

Compared to lower values in more anterior regions, we found greater FC and FDC in the splenium of the corpus callosum/forceps major in older adults compared to younger adults. Greater FC and FDC may reflect a greater diameter fiber, either in the size of the axon or myelination. The visual system, of which the forceps major is a part, develops early in life and has high levels of use and automaticity. A prior study found that myelination of the visual system is protracted beyond childhood and could extend into adulthood, which may partially explain our observed increase in FC and FDC in the forceps major (Meissner, Genç, Mädler, & Weigelt, 2019). Previous studies of aging and white matter have supported the last-in-first-out hypothesis which suggests that tracts most vulnerable to decline in aging mature and are myelinated later in development (Raz, 2000, 2001; Bender, Völkle, & Raz, 2016). Our findings along with previous results suggest that earlier-developing white matter pathways that support

consistently utilized, automated behavior (like forceps major of the visual pathway) may show protracted development in healthy older adults and exhibit greater protection from age-related declines.

It is also important to point out some of the study's limitations. One obvious limitation is that the data are cross-sectional rather than longitudinal. Some of the observed differences could therefore be due to cohort effects rather than within-person change as a result of age. Also, our sample of older adults were better educated than most and may not be representative of a less educated population. We also performed our analysis on single-shell b = 1000 s/mm^2 data, rather than multi-shell, higher b-value data which would have better angular resolution and an improved ability to account for partial volume effects. We are currently collecting multi-shell data for analysis in the future.

Despite these limitations, the present study demonstrates the power of fixel-based analysis in the study of differences in white matter between age groups. Not only can this kind of analysis shed light on the microstructural elements underlying differences in FA, it can also detect additional differences that could be missed by a DTI analysis.

One particularly striking feature of the fixel-based analysis results is that the major differences occur in white matter tracts that are associated with cognitive functions that are typically decline with age. For example, some of the most prominent differences were in the limbic white matter (Papez circuit), especially the fornix and cingulum. We also saw lower FDC in the temporal region of the parahippocampal cingulum. All of these tracts play a crucial role in episodic memory, which is typically worse in older compared to younger adults.

We also observed a significant asymmetry in the FBA results (particularly the FDC results), with differences being larger in more anterior regions relative to more posterior regions. This kind of anterior-posterior asymmetry is consistent with a number of previous DTI-based studies (Bennett et al., 2010; Bennett & Madden, 2014; Xie et al., 2016). We even observed this phenomenon within single tracts, such as the cingulum and the IFOF, where FDC was lower in the older group in the anterior but not posterior region. The varying differences along a single tract suggest different mechanisms of age-related effects in these regions, or different stages of a single, prolonged process. These findings support the idea of segment-specific effects of aging as described in Mårtensson et al. (2018) and Michielse et al. (2010). We also found significantly lower FC and FDC in the older group in white matter tracts in the prefrontal cortex. Many of these regions are thought to play a role in executive functions such as working memory, selective attention, and inhibition, all of which are typically worse in older adults.

Furthermore, the widespread lower values of FD, FC, and FDC presumably undermine the speed of neural transmission in general which might be expected to result in slower processing speed in older adults. And slower processing speed during behavioral tasks is a well-known feature of

aging, and has even been hypothesized to underlie some of the other behavioral deficits associated with aging (Salthouse, 1996).

DTI has been a useful tool for investigating white matter differences in age groups. However, its main metric FA is difficult to interpret, sensitive to multiple factors, and limited in its ability to capture complex microstructural phenomena. Here we used fixel-based analysis to investigate the complexities underlying differences in FA, but other methods such as diffusion kurtosis imaging (DKI) and neurite orientation and density imaging (NODDI) have also been used as well. One study used the various metrics of DTI, NODDI, DKI and multiexponential T2 relaxation to investigate microstructural differences in age groups and also found widespread white matter differences with regional variation of different measurements (Billiet et al., 2015). These results demonstrate the limitations of FA to describe complex microstructural phenomena and the power of employing other methods of white matter analysis alongside DTI to describe white matter differences associated with healthy aging.

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